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ALL DAY BLOOD SUGAR CURVES IN NONDIABETIC INDIVIDUALS AND IN DIABETIC PATIENTS WITH AND WITHOUT INSULIN *

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INTRODUCTION

Despite the many excellent studies on the use of insulin in diabetes, there has, as yet, been evolved no satisfactory routine method of regulating the dosage and the time of its administration. It is obviously to be desired that the doses be as small and the administrations as infrequent as possible. Moreover, with a diet adequate for maintenance and reasonable activity, the blood sugar concentration throughout the day must be held within normal limits. To accomplish this requires, first, an appreciation of what the normal blood sugar values throughout the day are and, second, an understanding of the effects that various diets and insulin dosages, and certain combinations of these, produce in diabetic patients. Only by intensive studies of the blood sugar concentration on large numbers of both normal persons and those in differing stages of the disease, can this knowledge be obtained. Our purpose, in this paper, is to present the results of such investigations, which we have made in this hospital during the last year, and to develop certain general rules for the use of insulin in the management of cases of diabetes. Incidentally, we will suggest a simple, practical method of estimating the full day blood sugar level in individual cases. First, blood sugar curves obtained from nondiabetic persons on adequate diets will be presented; then those from patients with diabetes of varying severity who were not being treated with insulin, and, finally, those from diabetic patients who were receiving insulin.

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MATERIAL AND METHODS

All the patients utilized were adults who had been under careful observation in the hospital for at least several days and were free of acidosis. The men were kept in a special metabolic ward while the women were in a general medical ward but closely watched. Certain nondiabetic patients served as controls. The food for all was prepared and weighed by a trained dietitian. For the sake of simplicity, as much as possible of the data relative to the individual patients is supplied in connection with the charts, and this is not repeated in the text.

The blood samples were removed from the veins at the elbow and analyzed by the method of Folin and Wu.[1] The specimens collected during the day were oxalated and analyzed immediately, while, during the night, with the exception of a few, they were collected in tubes containing sodium fluorid and thymol, as recommended by Sander,[2] and the determinations made the next morning. In a few of the earlier night collections, a drop of formaldehyd was added to the blood, and the analysis immediately carried to the stage where the water-clear filtrate was obtained. This filtrate was then placed in an icebox until the next morning, when the analysis was completed. Controls on both methods of preserving the specimens showed no change in the sugar concentration after they had stood from twelve to eighteen hours.

DESCRIPTION OF DATA

Nondiabetic Patients.—In Charts 1 to 6 are presented curves showing the hourly variations in blood sugar throughout twelve-hour periods on ward patients who were nondiabetic and had none of the other conditions commonly associated with hyperglycemia. As a group, it may be said of these that, with the food reasonably adequate and equally distributed among the three meals, the fluctuations in the concentration of blood sugar ranged from 0.075 to 0.155 per cent. It is also to be noted that the high points in the curves usually occurred one hour after the meals: the highest in three of the six, after breakfast; in two, after supper; in one, after lunch; and that there was a tendency for the curve to fall back to the fasting level within from one to two hours after each rise due to food.

Patients With Mild Diabetes Without Insulin.—Charts 7, 8 and 9 present curves from patients with mild diabetes who were not receiving any insulin but who had, for some time, been managed on dietary

1. Folin, O., and Wu, H.: A System of Blood Analysis; Simplified and Improved Method for Determination of Sugar, J. Biol. Chem. **41**:367-374 (March) 1920.

2. Sander, F. V.: Preservation of Blood for Chemical Analysis, J. Biol. Chem. **58**:1-15 (Nov.) 1923.

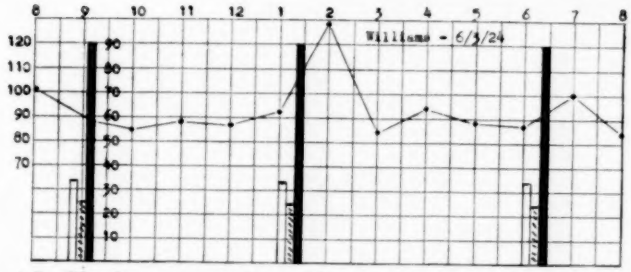

Chart 1.—Blood sugar curve in a white man, aged 49, weighing 58 kg., who entered the hospital, April 13, 1924, with typhoid fever; he was fully convalescent, going about the ward and on full diet at the time of the experiment. In this and in the following charts, the abscissas represent time intervals beginning in the forenoon, each space equaling thirty minutes, the figures at the top being hours; the ordinates represent milligrams of blood sugar per hundred cubic centimeters of blood (or thousandths of 1 per cent.), each space equaling 10 mg. Such spaces also represent 10 gm. of food and 10 units of insulin where these are indicated. The food is presented in grams of protein, fat and carbohydrate; the protein by an open column; the fat by a hatched one, and the carbohydrate by a solid one. In all instances, the time of administration is indicated by the left border of the protein column; the insulin, by a narrow, solid, perpendicular line, and chart numbers correspond to the curve numbers in the text.

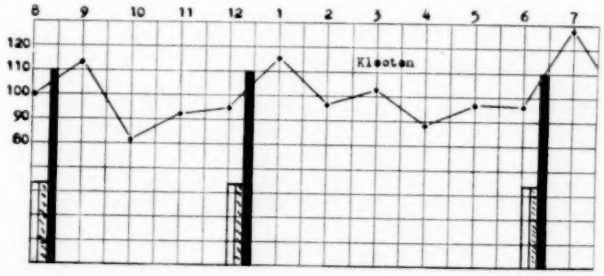

Chart 2.—Blood sugar curve in a white man, aged 35, weighing 52.5 kg., confined in the hospital from July 16 to Aug. 6, 1924; diagnosis, gastric neurosis (hyperacidity).

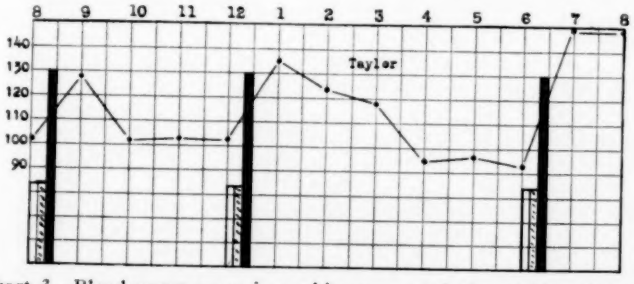

Chart 3.—Blood sugar curve in a white man, aged 63, weighing 50 kg., confined in the hospital from July 16 to Aug. 2, 1924; diagnosis, arthritis deformans. He had had symptoms for two years. There was slight secondary anemia.

restriction alone. It will be noted that in Cases 7 and 8, the fasting blood sugar levels were somewhat high (0.145 and 0.147 per cent.); while in Case 9, the value was only 0.100 per cent. The former two present a type of curve that we have found common to most diabetic

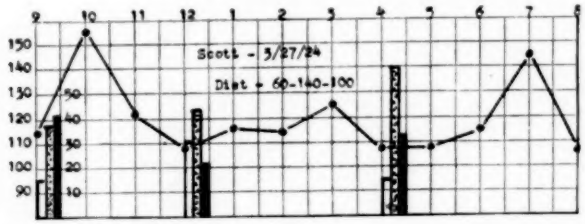

Chart 4.—Blood sugar curve in a white man, aged 29, weighing 57 kg., who was convalescent from lobar pneumonia, and in a good general condition.

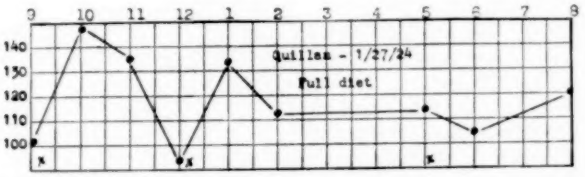

Chart 5.—Blood sugar curve in a white man, aged 43, weighing 67 kg., who entered the hospital, Jan. 12, 1924; diagnosis, chronic appendicitis; *X* indicates full house diet consisting of, approximately, 90 gm. of protein, 70 gm. of fat and 200 gm. of carbohydrate.

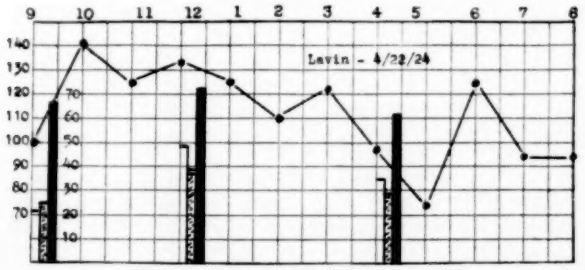

Chart 6.—Blood sugar curve in a white girl, aged 19, weighing 62 kg., who entered the hospital, April 7, 1924, because of pain in right hip and thigh; diagnosis, myositis.

patients, in that the high points occur after the first and last meals of the day, the highest after breakfast, there being only an insignificant rise after the midday meal. This is seen in only one of our nondiabetic patients (Chart 4). Both these curves are on a higher level throughout

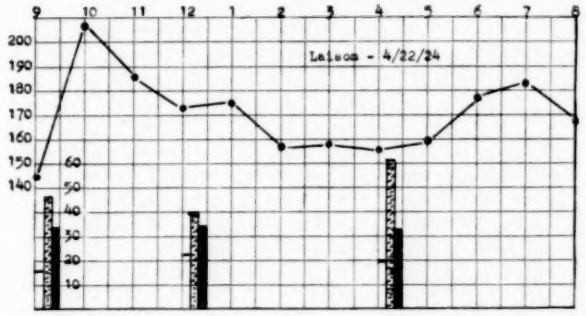

Chart 7.—Blood sugar curve in a white woman, aged 45, weighing 64 kg., who had had symptoms for several years. She entered the hospital, March 28, 1924, showing glycosuria, no ketonuria, and a fasting blood sugar of 0.187 per cent. No insulin had been given.

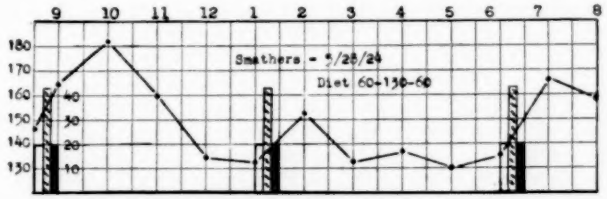

Chart 8.—Blood sugar curve in a white man, aged 65, weighing 106 kg., who had had symptoms for five years. He was admitted to the hospital, March 27, 1924, at which time he showed glycosuria, no ketonuria, a fasting blood sugar concentration of 0.268 per cent., and a plasma carbon dioxid content of 55 per cent. by volume. Insulin therapy was begun April 1, but had been stopped before this experiment was made. Later, as the diet was increased, insulin had to be resumed.

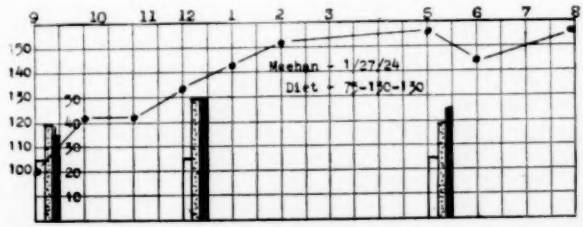

Chart 9.—Blood sugar curve in a white man, aged 55, weighing 75 kg., who had had symptoms for five years. He was admitted, Jan. 10, 1924, showing a trace of sugar in the urine, no diacetic acid, and a fasting blood sugar of 0.130 per cent. No insulin had been given.

the day than those of our nondiabetic patients. In these cases, the carbohydrate in the diet was equally divided among the three meals, though the fat content in Case 7 was increased in the evening. In Case 9, the curve is different, being only slightly elevated after the morning meal, and steadily rising throughout the rest of the day; but here the carbohydrate fraction of the diet was greater at the two later meals. The diets in all of these cases were fairly adequate, and yet in Cases 7 and 8, the blood sugar concentration exceeded the accepted threshold value for glycosuria (0.170 per cent.) only after breakfast. It would seem, therefore, that these were suitable cases for a small dose of insulin in the early morning.

Patients With Severe Diabetes Without Insulin.—The patients whose curves are presented in Charts 10 and 11 may reasonably be considered severe, or potentially severe, diabetic ones since their fasting blood sugar concentrations at the time of admission were 0.288 and 0.300 per cent., respectively, in spite of the fact that neither showed ketonuria. The absence of the latter was undoubtedly due to the fact that both had been carefully dieted before admission to the hospital. Their curves resemble in type those of our milder Cases 7 and 8, though at a decidedly higher level. The high points reached after breakfast and supper are striking, particularly in Case 11. It is true that the carbohydrate factor in the lunch of this patient was reduced to about one-half that of the breakfast, whereas in Case 10 it was increased, but this, while it would perhaps explain the difference between the after lunch reaction in these two special cases, would not seem a sufficient explanation for the general type of the curve. This will be discussed later.

Patients with Mild Diabetes with Insulin.—The curves in Charts 12 to 21 have been obtained from mild to fairly severe diabetic patients who were receiving insulin in moderate quantities. The first three cover full twenty-four hour periods, the food intake being the same at each meal and identical for the three patients. The insulin dosage also was the same, 10 units one-half hour before each meal. The effect of the morning insulin dosage was to reduce slightly the blood sugar concentration within the half-hour before breakfast, this reduction being promptly overcome by the food. In Cases 12 and 13, there was a definite rise in the sugar concentration within from one to two hours after each meal, highest in the morning and least marked after the midday meal, as in two of our mild and one of our severe cases without insulin. It is striking that this was not true in Case 14, there occurring only a slight rise after breakfast and then a steady drop throughout the day, in spite of food. This result aroused our interest, and we requested roentgenologic study of the gastro-intestinal tract of this patient to

determine if there was any indication of delayed emptying of the stomach which might account for delayed absorption. We were surprised to receive a report to the effect that the patient had evidence of some foreign substances in the stomach, possibly benign tumors, and that these did interfere with gastric evacuation.

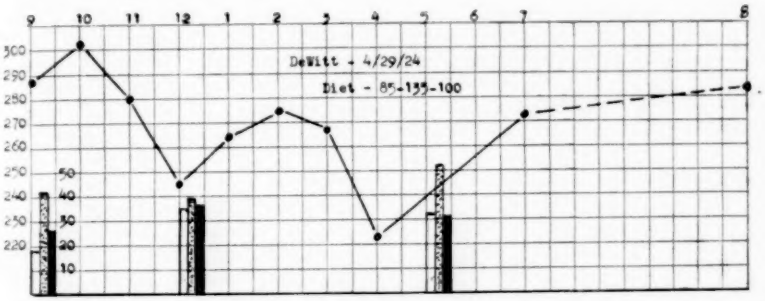

Chart 10.—Blood sugar curve in a white man, aged 56, weighing 74 kg.; a case of acromegaly. He had shown a tendency to glycosuria for seven years, but this was controlled most of that time by careful dieting. He had received no insulin. On his admission to the hospital, April 28, 1924, the urine contained sugar but no ketones and the fasting blood sugar amounted to 0.288 per cent.

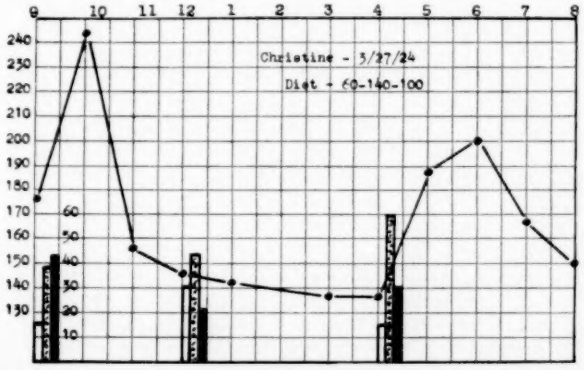

Chart 11.—Blood sugar curve in a white woman, aged 42, weighing 66.5 kg., with symptoms for two years. She entered the hospital, March 3, 1924, at which time she had glycosuria, no ketonuria and a blood sugar of 0.300 per cent. Insulin was begun, March 15. She was discharged, April 8, aglycosuric, on a diet of 60 gm. of protein, 140 gm. of fat and 100 gm. of carbohydrate, with insulin, 15 units, before breakfast.

These three curves (Charts 12, 13 and 14) were the first ones we made, and the similarity of the gradual rise during the night to practically the fasting level of the previous morning suggested that it was not necessary to follow all our cases throughout the night.

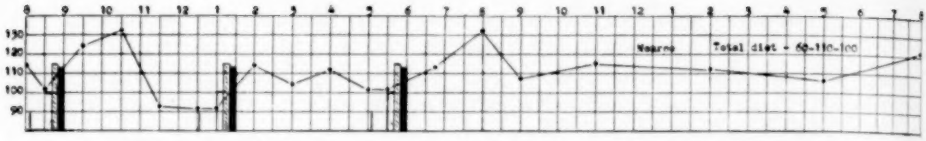

Chart 12.—Blood sugar curve in a white man, aged 43, weighing 56 kg., with symptoms for two years. He entered the hospital, Oct. 29, 1923, showing sugar but no ketones in the urine, a fasting blood sugar of 0.169 per cent., and a plasma carbon dioxid content of 62 per cent. by volume. Insulin was begun, November 10. He was discharged, November 30, showing no sugar or ketones in the urine, on a diet of 60 gm. of protein, 125 gm. of fat, 125 gm. of carbohydrate and 10 units of insulin before each meal.

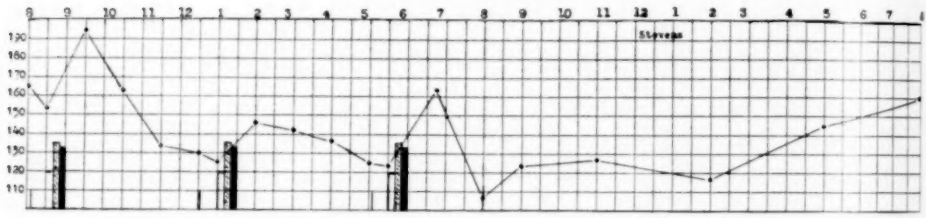

Chart 13.—Blood sugar curve in a white man, aged 23, weighing 50 kg., with symptoms of four and one-half years' duration. He entered the hospital, Nov. 7, 1923, showing glycosuria, ketonuria, a fasting blood sugar of 0.244 per cent. and a plasma carbon dioxid content of 30 per cent. by volume. Insulin was begun on the second day. He was discharged, December 12, showing no sugar or ketones in the urine on a diet of 60 gm. of protein, 150 gm. of fat and 150 gm. of carbohydrate, while taking insulin, 10 units, before each meal.

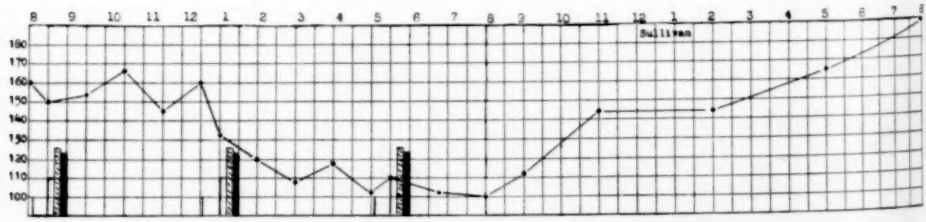

Chart 14.—Blood sugar curve in a white man, aged 54, weighing 55 kg., who had had recognized diabetes for eight years. He entered the hospital, Nov. 9, 1923, showing glycosuria, no ketonuria, a fasting blood sugar of 0.268 per cent. and a plasma carbon dioxid content of 58 per cent. by volume. Insulin therapy was begun, November 15. He was discharged, November 30, without sugar or ketones in the urine, on a diet of 60 gm. of protein, 110 gm. of fat and 120 gm. of carbohydrate, and taking insulin, 10 units, before each meal.

In the next case (Chart 15), only a morning dose of 10 units of insulin was given with the breakfast, and it will be noted that there occurred no rise after the midday meal, but a distinct, though slow, one after the evening meal. The same occurred in Cases 16 and 17, though 20 units of insulin were used. To have prevented the evening rise, a second dose would seem to have been indicated. Consequently, in Case

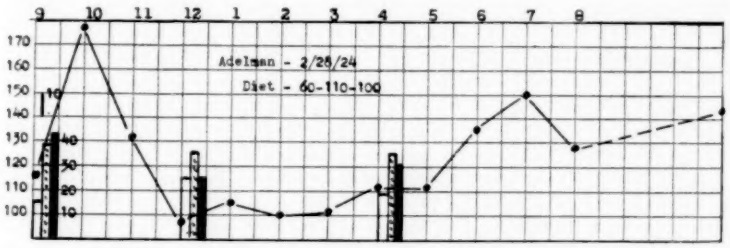

Chart 15.—Blood sugar curve in a white man, aged 52, weighing 83 kg., with symptoms for eight years. He entered the hospital, Feb. 9, 1924, showing glycosuria, no ketonuria and a fasting blood sugar of 0.206 per cent. Insulin was begun February 24. He was discharged, March 3, sugar free, on a diet of 60 gm. of protein, 110 gm. of fat and 100 gm. of carbohydrate, with 10 units of insulin before breakfast.

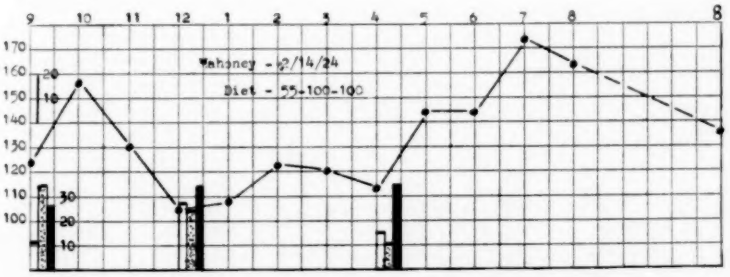

Chart 16.—Blood sugar in a white woman, aged 38, weighing 94 kg., with symptoms for one year. She entered the hospital, January 27, at which time she had a fasting blood sugar of 0.221 per cent. and a plasma carbon dioxid content of 67 per cent. by volume, but no glycosuria on a full diet. No insulin had been used.

18, somewhat more severe, we gave, in addition to 20 units of insulin in the morning, an evening dose of 10 units. There resulted no sharp rise in the evening curve, but a steady one, over four hours, to a point slightly higher than the morning level, and this occurred in spite of a reduction in the amount of carbohydrate at the supper. This second dose should, therefore, have been a larger one.

Cases 19 and 20, which were still more severe (fasting levels 0.182 and 0.196 per cent.), were given larger morning doses of insulin (25 units) but, at the same time, more carbohydrate (65 gm. each) at breakfast, and the insulin was given half an hour before the food. In

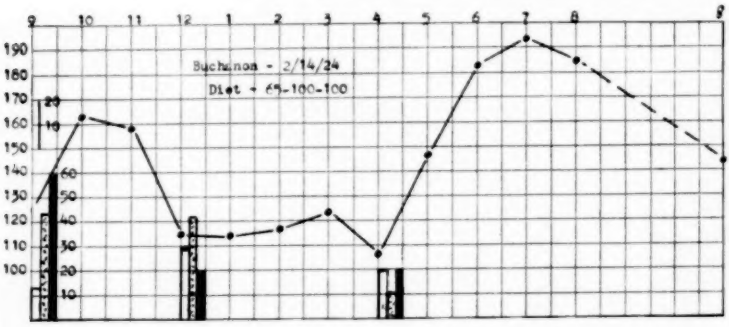

Chart 17.—Blood sugar curve in a white woman, aged 50, weighing 68.5 kg., with symptoms for two years. She entered the hospital, Jan. 16, 1924, at which time she had glycosuria, no ketonuria, a fasting sugar reading of 0.224 per cent., and a plasma carbon dioxid content of 65 per cent. by volume. Insulin was begun one week later. She was discharged February 16, sugar free on insulin, 10 units, twice daily, and a diet of 60 gm. of protein, 135 gm. of fat and 120 gm. of carbohydrate.

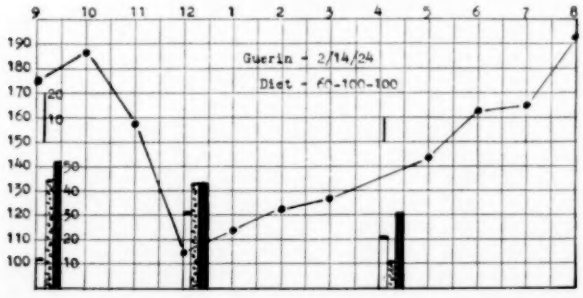

Chart 18.—Blood sugar curve in a white woman, aged 67, weighing 71 kg., with symptoms for twelve years. She entered the hospital, Dec. 18, 1923, because of gangrene of the toe, at which time she had glycosuria, no diacetic acid, a fasting blood sugar of 0.208 per cent., and a plasma carbon dioxid content of 56 per cent. by volume. She had been untreated until admission. Insulin therapy was begun, December 20.

neither of these did the rise in the sugar concentration after breakfast reach the fasting level, thus suggesting an advantage in giving the insulin a short time before the meal. Here, again, it will be noted that there occurred no rise immediately following the luncheon, though no special

significance can be attached to this in these two instances, since the carbohydrate factor in the midday meal was relatively small. Later in the afternoon, there was a distinct rise; so another dose of insulin was indicated before supper.

The curve in Chart 21 is from a fairly severe diabetic patient who had been improving steadily under insulin. It was planned to give him

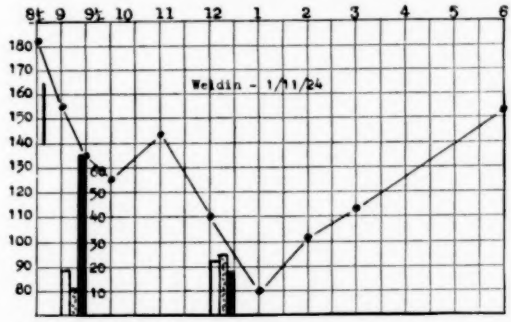

Chart 19.—Blood sugar curve in a white man, aged 34, weighing 43 kg., with symptoms for four months. He entered the hospital, Dec. 30, 1923, at which time he showed glycosuria, ketonuria, a fasting blood sugar of 0.345 per cent. and a plasma carbon dioxid content of 50 per cent. by volume. Insulin was begun, Jan. 3, 1924.

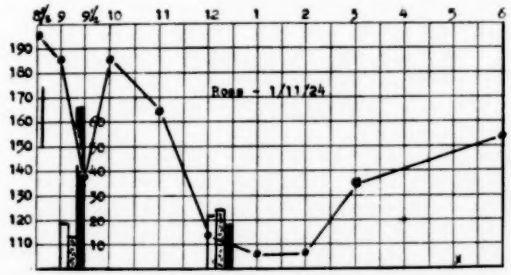

Chart 20.—Blood sugar curve in a white man, aged 39, weighing 57 kg., with symptoms for two years. He had had furuncles for three weeks before admission. He entered the hospital, Dec. 9, 1923, at which time he was glycosuric, ketonuric, and had a fasting blood sugar reading of 0.283 per cent. and a plasma carbon dioxid content of 31 per cent. by volume. Insulin therapy was begun on the second day. He was discharged, Jan. 20, 1924, with no sugar or diacetic acid in the urine and on a diet of 55 gm. of protein, 170 gm. of fat, 80 gm. of carbohydrate, and 30 units of insulin before breakfast.

only 15 units the day of our experiment, but by mistake he received 40 units. We therefore increased his carbohydrate, a half-hour later, to 50 gm. (twice the amount it had been intended he should have), and gave the same amount at luncheon, thus securing the very satisfactory curve which is presented.

In reviewing this group, it will be observed that when the insulin was given at the same time as the breakfast, a rise in the blood sugar concentration developed after this meal. Even when only 10 gm. of carbohydrate was given with 40 units of insulin, as in Case 21, there occurred no immediate fall, and a moderate rise after the regular breakfast, thirty minutes later. In those, however, in which the insulin was given alone one-half hour before breakfast, a prompt decline in the blood sugar value developed, and the peak of the rise which followed the meal was usually below the fasting level. Thus, it would seem clear that to avoid the excessive hyperglycemia incident to the first meal of the day in these mild cases, the insulin should be given at least thirty minutes before the food.

Patients with Severe Diabetes with Insulin.—It is not so satisfying to present the remaining cases, those of severe diabetes with insulin, because a practical method of securing curves approaching the normal,

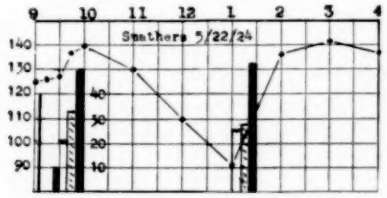

Chart 21.—Blood sugar curve in a white man, aged 25, weighing 106 kg., with symptoms for five years. He entered the hospital, March 27, 1924, at which time he showed glycosuria, no ketonuria, a fasting blood sugar of 0.268 per cent., and a plasma carbon dioxid content of 55 per cent. by volume. Insulin therapy was begun, April 1. He was discharged, May 29, sugar free, taking a diet of 55 gm. of protein, 160 gm. of fat and 80 gm. of carbohydrate, with no insulin.

in a routine manner, has not been evolved. We feel, however, that our curves are enlightening and may eventually lead to more satisfactory results.

In certain cases of this group, only one large daily dose of insulin was given in an attempt to determine if it were possible to treat efficiently these patients with the one dose a day; this was given immediately before breakfast, and most of the carbohydrate allowed for the day was given at that meal. Chart 22 shows two curves of a patient who was being managed in this way, with an interval of one week between them. Only a morning dose of insulin was given each day, 35 units the first time and 40 the second, and it was administered immediately before breakfast. The improvement that occurred during the week, as shown in the general level of the curves, is manifest. The fasting levels were not exceeded during the twenty-four hours, and there was no rise after the

first two meals of the day. After the supper, there occurred a steady rise until the following morning. The curve from Case 23 shows the same features. In another patient (Charts 24 and 25) the curves, with an interval of a week between them, show similar characteristics, including a great improvement in the general level during the week, but in these there developed a rise after breakfast. In view of our results in the milder cases, it was thought that, had the insulin in this instance been given from a half to an hour earlier, this postbreakfast rise might

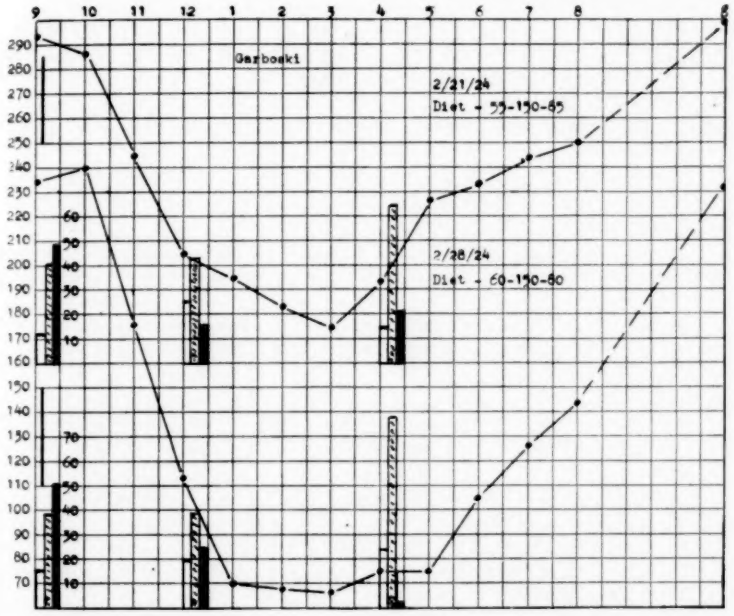

Chart 22.—Blood sugar curve in a white man, aged 32, weighing 55 kg., with symptoms for one year. He entered the hospital, Feb. 1, 1924, at which time he had glycosuria, ketonuria, a fasting blood sugar of 0.332 per cent., and a plasma carbon dioxid content of 45 per cent. by volume. Insulin was begun February 5.

have been avoided. Even such timing of the insulin dosage is not always sufficient to overcome the rise, however, as is illustrated by Case 25 (Charts 26 and 27). On the other hand, the insulin (40 units) was given to another patient (Chart 28) a half-hour before breakfast, and the subsequent rise was eliminated. So it would seem impossible, in the individual severe case, to predict whether or not a dose of insulin which is sufficient to bring the sugar concentration down to a reasonably

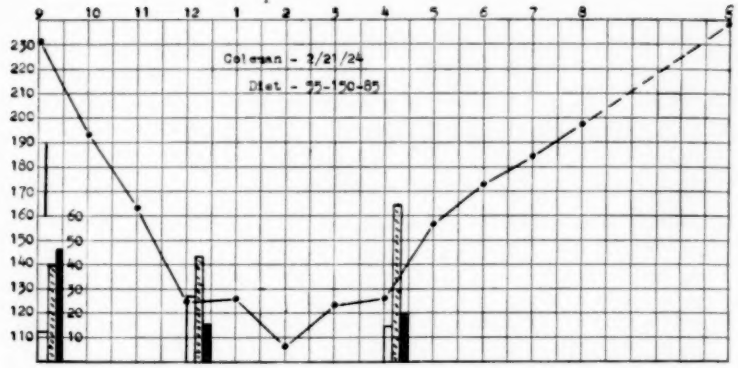

Chart 23.—Blood sugar curve in a white man, aged 32, weighing 43.7 kg., with symptoms for five months. He entered the hospital, Feb. 6, 1924, at which time he had glycosuria, ketonuria, a fasting blood sugar of 0.210 per cent., and a plasma carbon dioxid content of 39 per cent. by volume. He had numerous furuncles. Insulin was begun three days after admission. He was discharged, February 25, aglycosuric on a diet of 55 gm. of protein, 150 gm. of fat, and 80 gm. of carbohydrate, with insulin, 30 units before breakfast and 10 units before supper.

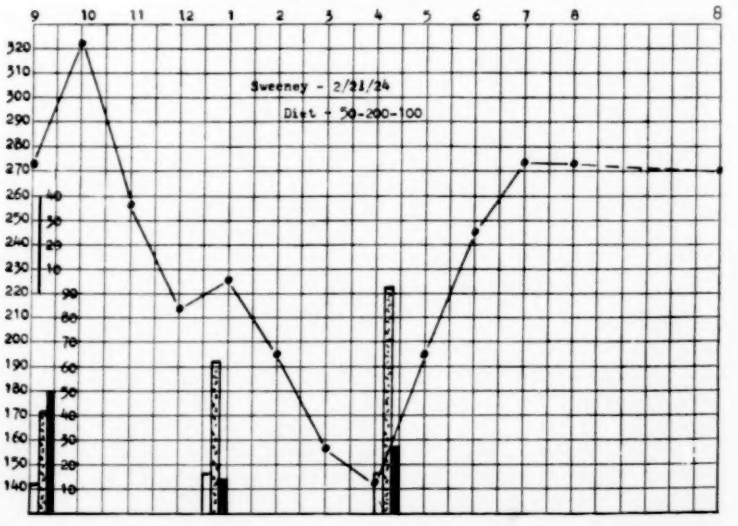

Chart 24.—Blood sugar curve in a white man, aged 36, weighing 52 kg., with symptoms for fourteen months. He entered the hospital, Feb. 7, 1924, at which time he showed glycosuria, a fasting blood sugar of 0.222 per cent. and a plasma carbon dioxid content of 57 per cent. by volume. He had taken insulin before admission.

low level by the middle of the day will prevent a rise within an hour after breakfast, be it given immediately before or from a half to one hour before the first meal of the day. It seems justifiable, however, in

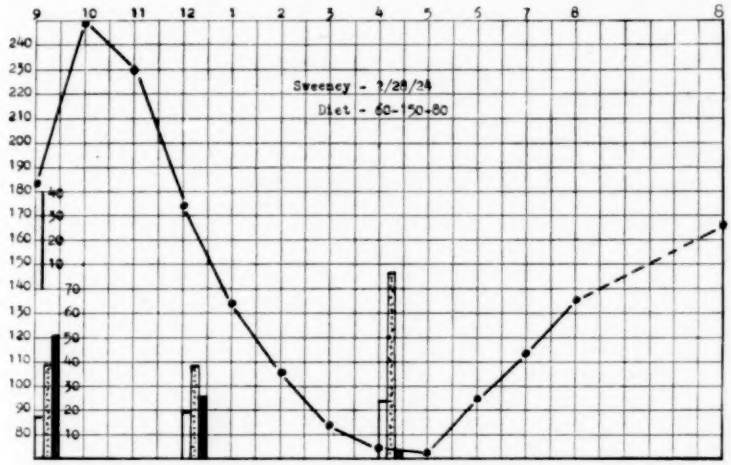

Chart 25.—Blood sugar curve in same patient as Chart 24; improvement in general level of the curve after the lapse of one week.

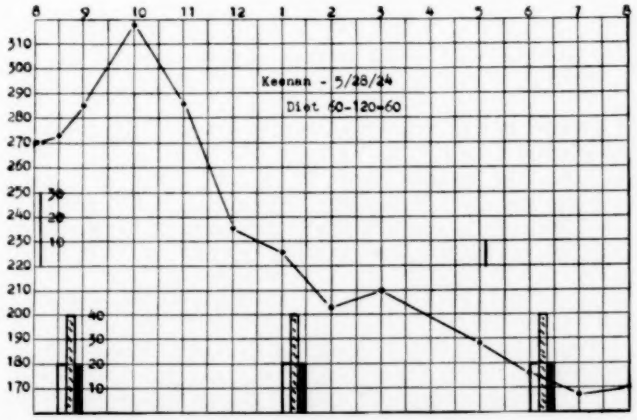

Chart 26.—Blood sugar curve in a white woman, aged 53, weighing 54 kg., with symptoms for two years. She entered the hospital, May 6, 1924, at which time she showed glycosuria, no ketonuria and a fasting blood sugar of 0.329 per cent. Insulin was begun May 16.

the light of our experience with the milder cases, to believe that the half to one hour interval between the insulin and the food is more effective in this respect.

In Case 26 (short curve of Chart 28), as well as in Case 21, the experiment was made by giving 10 gm. of glucose at the same time as the insulin (40 units) and the regular meal a half-hour later to determine how this might affect the sugar concentration curve. It was thought that, in some

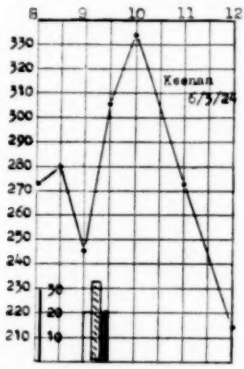

Chart 27.—Blood sugar curve in same patient as Chart 26.

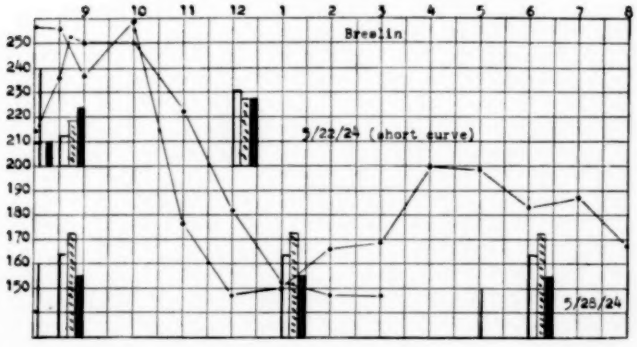

Chart 28.—Blood sugar curve in a white man, aged 29, weighing 50 kg., with symptoms for two years. He entered the hospital, May 10, 1924, showing glycosuria, ketonuria, a fasting blood sugar of 0.463 per cent., and a plasma carbon dioxid content of 15 per cent. by volume. He left the hospital, against advice, June 7, with a trace of glycosuria, on a diet of 60 gm. of protein, 100 gm. of fat, and 50 gm. of carbohydrate, and insulin, 35 units before breakfast and 25 units before supper.

way, this small amount might initiate the mechanism of glucose disposal and so prevent the excessive rise in blood sugar after the larger amount of carbohydrate given with the breakfast. It will be noted, however, that even this small dose of carbohydrate itself caused a definite rise within thirty minutes, in spite of the large accompanying injection of

insulin, and that in Case 26 the level was still higher fifteen minutes later, after the breakfast had been taken. The uselessness of this procedure is therefore suggested.

The curve of Case 27 (Chart 29) resembles others of this group, except that the second dose of insulin, in the evening, was sufficient to prevent a rise in the level after supper, as it was also in Case 26 (long curve). The morning dose in Case 27, however, was obviously not large enough for the severity of the case and the amount of carbohydrate allowed.

These data show that, in the more severe cases, it is possible to bring the blood sugar level down to a reasonably satisfactory figure by midday and to keep it there during most of the afternoon by giving a large dose of insulin one-half to an hour before breakfast. A second dose in the

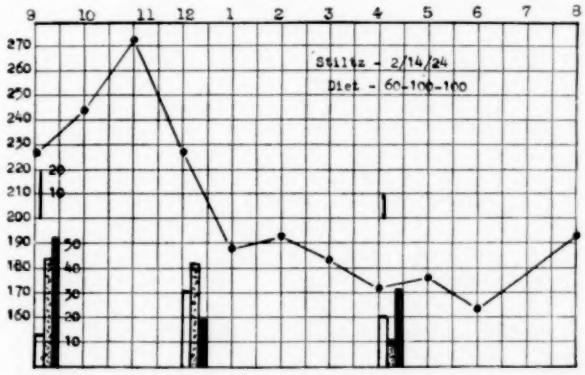

Chart 29.—Blood sugar curve in a white woman, aged 62, weighing 44 kg., with symptoms for six years. She entered the hospital, Jan. 20, 1924, at which time she showed glycosuria, ketonuria, a fasting blood sugar of 0.440 per cent., and a plasma carbon dioxid content of 35 per cent. by volume. Insulin therapy was begun at once. She was discharged, April 6, still showing traces of sugar in the urine, no ketonuria, a fasting blood sugar of 0.300, and a plasma carbon dioxid content of 59 per cent. by volume. At this time, she was on a diet of 80 gm. of protein, 140 gm. of fat and 100 gm. of carbohydrate, and getting insulin, 20 units before breakfast and 10 units before supper.

late afternoon, provided it is of sufficient amount, will prevent any marked rise after supper. It was frequently observed, however, that such treatment did not prevent a slow rise during the night to an unsatisfactory level by the following morning.

To overcome this excessive morning concentration, we have, in certain instances, administered a third dose of insulin at midnight, thus giving it at practically eight-hour periods. Charts 30, 31 and 32 show the effects of such timing of the insulin dosages. The curve in Chart 30 was made of a patient who had been showing glycosuria and high morn-

ing fasting figures consistently on a fixed diet and with adequate morning and evening doses of insulin. On the day of the experiment (no insulin having been given during the preceding night), with the same diet and the same two day doses of insulin, a third midnight dose (10 units) was added. It will be observed that, as a result, the concentration on the following morning was only 0.131 per cent., and that the curve was practically flat throughout that night. Chart 31 shows similar features. It is unfortunate that we could not have continued these curves for another twelve hours for comparison with the previous day. If one started with the low morning level, it seems probable that a very satisfactory result throughout the twenty-four hours would

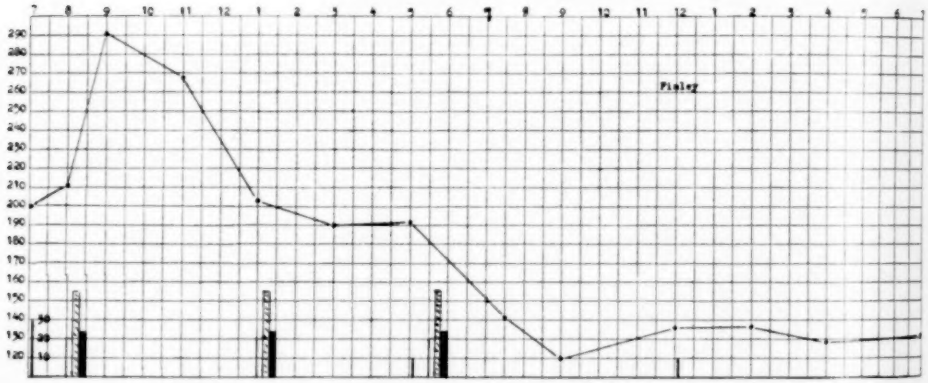

Chart 30.—Blood sugar curve in a white woman, aged 70, weighing 55 kg., with diabetes of five years' duration. She was admitted, June 3, 1924, with glycosuria, mild ketonuria, a blood sugar of 0.352 per cent., and a plasma carbon dioxid content of 53 per cent. by volume. Insulin therapy was begun, June 9. She was discharged, July 16, at which time she was taking 60 gm. of protein, 130 gm. of fat, 70 gm. of carbohydrate, and 30 units of insulin before breakfast, 10 before supper and 10 at midnight. On this regimen, she became free of ketones and sugar in the urine, and had a fasting blood sugar of 0.122 per cent.

have been obtained. This is proved, apparently, by the fact that the patients on this regimen became free of sugar in the urine. There were at no time any symptoms of hypoglycemia.

Finally, a curve was obtained from another patient (Chart 32) who had been on three doses of insulin daily, so timed, for a week, and who in consequence had become aglycosuric. On admission to the hospital, she was practically in diabetic coma. A few days later, her fasting blood sugar level was 0.262 per cent., and the peak of the rise, after breakfast, with a preceding dose of 25 units of insulin, was 0.327 per

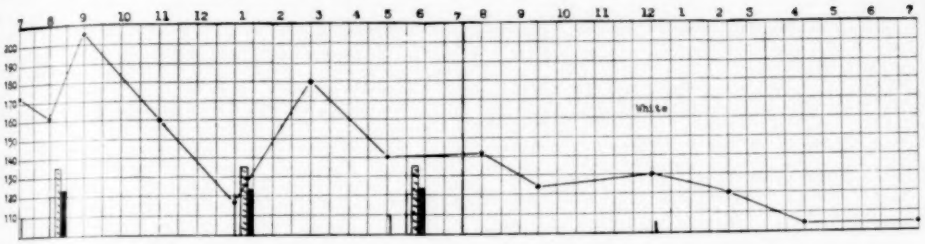

Chart 31.—Blood sugar curves in a white man, aged 57, weighing 49 kg., with symptoms for ten months. The Wassermann reaction was positive. On admission to the hospital, the urine showed sugar, acetone and diacetic acid; the blood sugar was 0.232 per cent., and the plasma carbon dioxid content, 40 per cent. by volume. With three doses of insulin daily, as shown in this chart, the urine became sugar free, and later continued to be sugar free on two doses daily, 15 units before breakfast and 10 before supper. On discharge, the fasting blood sugar level was 0.115 per cent.

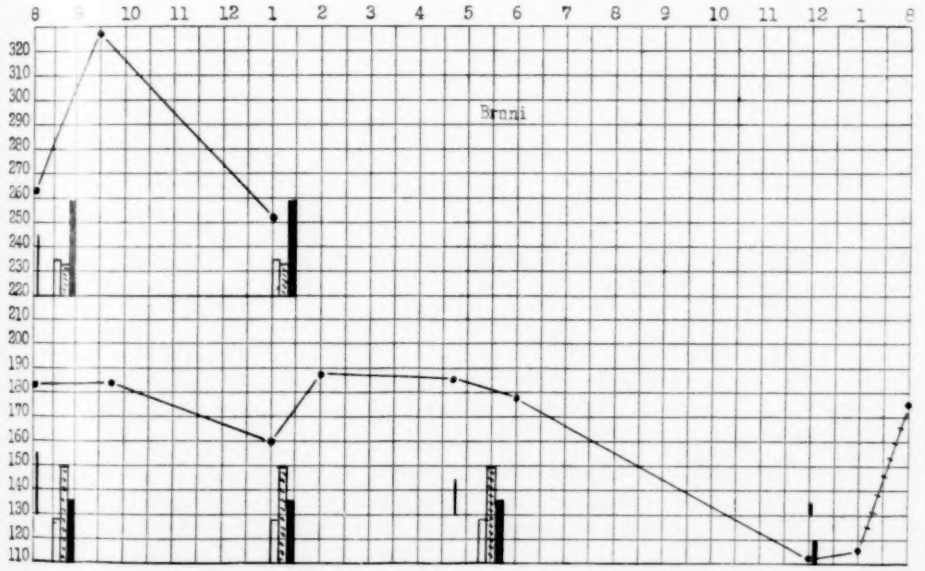

Chart 32.—Blood sugar curve in a white woman, aged 34, weighing 53 kg., with symptoms of diabetes for four years. She was treated in several hospitals, and had had insulin for a time, but had stopped it before admission to the University Hospital. On admission, she was practically in coma, though slightly responsive to commands. She had a blood sugar concentration of 0.548 per cent. and a plasma carbon dioxid content of 7 per cent. by volume. Ketonuria was marked. She came out of coma after the administration of 180 units of insulin and glucose, intravenously, during the first twenty-four hours. Three days later, when on diet of 45 gm. of protein, 35 gm. of fat, 115 gm. of carbohydrate and insulin, 60 units, the curve in the upper part of the chart was made. A week later, the lower curve was made. She was then entirely free of both sugar and ketones in the urine.

cent. (upper curve in Chart 32). At this time, we began to give 10 units of insulin at midnight, without any food, later increasing the dosage to 15 units. Then he became sleepless, had a little sweating and a sensation of hunger. To overcome these symptoms, 10 gm. of carbohydrate were given at the same time as the night insulin, and, finally, the latter was reduced to 5 units, but the carbohydrate feeding was continued. The hypoglycemic manifestations were in this way promptly controlled. The curve presented shows no excessive hyperglycemia on a diet containing 50 gm. of protein, 115 gm. of fat and 110 gm. of carbohydrate. At the same time, she was getting a total of only 45 units of insulin: 25 units a half-hour before breakfast, 15 units a half-hour before supper, and 5 units at midnight. The lowest blood sugar value (0.112 per cent.) was present at midnight, suggesting that the dose of insulin before supper might safely have been somewhat reduced. We have not obtained such a marked reduction in the fasting blood sugar level so quickly without the use of insulin during the night.

COMMENT

The response of the human organism to the ingestion of a single dose of carbohydrate, as determined by the sugar concentration of the blood for from two to four hours afterward, has been extensively studied in both normal and diabetic subjects, but sufficient attention has not been directed to the reaction that follows repeated meals of mixed composition throughout full day periods. Such studies are of great practical importance in connection with the administration of insulin to patients with diabetes.

It is of course fully appreciated that ordinarily, unless insulin has been administered beforehand, a rise in the blood sugar level follows every meal containing carbohydrate. That diets free of carbohydrate have no such effect in normal persons is also understood. This has been demonstrated by several investigators, among them Mosenthal, Clausen and Hiller,[3] who showed, however, that in patients with mild diabetes there sometimes occurred a slight rise after meals containing only very small amounts of carbohydrate. These aspects of the problem have not been touched on in our work; neither have we studied the effects of insulin alone, without food, on the blood sugar concentration, because this has been adequately presented by Banting, Best, Collip, Campbell and Fletcher [4] and by Fletcher and Campbell.[5] Their curves

3. Mosenthal, H. O.; Clausen, S. W., and Hiller, A.: The Effect of Diet on Blood Sugar, Arch. Int. Med. **21**:93 (Jan.) 1918.

4. Banting, F. G.; Best, C. H.; Collip, J. B.; Campbell, W. R., and Fletcher, A. A.: Pancreatic Extract in Diabetes, Canad. M. A. J. **12**:141-146 (March) 1922.

5. Fletcher, A. A., and Campbell, W. R.: The Blood Sugar Following Insulin Administration, J. Metabolic Res. **2**:637-649 (Nov.-Dec.) 1922.

were plotted to show hourly variations in the blood sugar over periods of from four to seven hours and the values at less frequent intervals for a longer time. As is now well known, they found a steady fall in the curve, which reached its low point at from two to twelve hours, usually four, and return to the original level at a variable rate. The general effects were found to be the same in nondiabetic patients and in diabetic patients, but there was a tendency for the decline to be dependent on the initial blood sugar concentration, being greater with the higher fasting level.

Our interest has centered mainly on the effects of three meals of mixed composition, on the level of blood sugar throughout the day in diabetic patients, and on the modifying influence of insulin administrations. In our work, it soon became apparent that the effect was not the same for each meal of the day, whether nondiabetic or diabetic patients were being studied, and whether or not insulin was being given. The general tendency, with three meals of equal composition, was for the maximal rise to occur after breakfast. Mosenthal, Clausen and Hiller had noted this in their mild diabetic patients who were on diets largely free of carbohydrate. When large amounts of carbohydrate were given to one of their diabetic patients, however, they got a different result, the blood sugar figures after the noon and evening meals being greater than after breakfast. From this single case, they inferred that the course of events in diabetic patients with a high carbohydrate diet was different. It must be pointed out, however, that the amounts of carbohydrate given at the later meals in their case were greater than the amount given at breakfast (116 and 75 gm. as against 45). Our results, as seen in many cases of both mild and severe diabetes in which the patients received the same amount of carbohydrate at each meal, indicate that the maximal rise usually occurs after the breakfast, even when an adequate amount of carbohydrate is allowed. It would seem, therefore, that this holds true for diets either low or reasonably high in carbohydrates.

Hamman and Hirschman[6] have observed in normal persons that superimposed doses of glucose are not necessarily always followed by a steplike rise in the blood sugar, but frequently by a less marked reaction or none at all; and they concluded from their experiments that the ingestion of glucose in some way stimulated the mechanism of carbohydrate disposal so that the repeated ingestion of the same amount caused a less marked hyperglycemia. Similar results were obtained by

6. Hamman, L., and Hirschman, I. I.: Effects Upon the Blood Sugar of the Repeated Ingestion of Glucose, Bull. Johns Hopkins Hosp. **30**:306 (Oct.) 1919.

MacLean and de Wesselow,[7] who gave the glucose doses at one and one-half hour intervals. Their curves after the first and the second doses had the same form, but the first one was distinctly more elevated. The explanation they give is that the hyperglycemia resulting from the first dose initiates the glycogen-forming mechanism which causes a fall in the sugar level, and that this mechanism, not having subsided when the second one is given, tends to prevent so marked a blood sugar concentration after that dose. In diabetes, they feel that this mechanism fails in varying degree. Foster [8] also has presented curves showing a less decided reaction from a second dose of glucose in normal persons, and has adduced evidence that seems very convincing in favor of the theory advanced by MacLean and de Wesselow. This hypothesis has been questioned, however, by Folin and Berglund [9] who believe that the fall comes when the tissues are well supplied with available food material, and that the lower level is simply an index of the fact that the need for sugar transportation from some tissues to others has temporarily dropped very low or ceased altogether.

9. Folin, O., and Berglund, H.: Transportation, Retention and Excretion of Carbohydrates, J. Biol. Chem. **51**:213-273 (March) 1922.

Whatever the true explanation may be, our only immediate interest is in the fact that under certain circumstances, in both nondiabetic and diabetic patients, a second dose of carbohydrate, given within a few hours after the first, does not cause such a high blood sugar rise as the former one. This is seen in many of our cases in which no insulin was used (Charts 4, 5, 6, 7, 8, 10 and 11), including both nondiabetic and diabetic. It also occurred in many of the cases in which insulin was given, but in these the insulin itself was probably the main factor.

It seems essential, however, in order to secure this lessened rise after a second dose, that not too long a period elapse between the feedings. MacLean and de Wesselow found that in normal persons an interval of three or four hours was sufficient for the sugar concentration again to reach the fasting level and that the reaction to the second dose was independent of the first one. It may be assumed, according to their theory, that in this length of time the glycogen-forming mechanism subsides. This would seem to explain those of our cases in which the rise after luncheon or the evening meal was equal to or greater than that after breakfast (Charts 1, 2 and 3). Our data would suggest that this subsidence of glycogen formation occurs more readily in the nondiabetic than in the diabetic patient.

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7. MacLean, H., and de Wesselow, O. L. V.: Estimation of Sugar Tolerance, Quart. J. Med. **14**:103 (Jan.) 1921.

8. Foster, G. L.: Carbohydrate Metabolism; Interpretation of Blood Sugar Phenomena Following Ingestion of Glucose, J. Biol. Chem. **55**:303-314 (Feb.) 1923.

Our six twelve-hour curves on nondiabetic patients with mixed diets show characteristics that can be reconciled with the foregoing considerations. In practically all, there occurred a rise in the blood sugar level after each meal. When the interval between breakfast and luncheon was only three hours, the rise after the latter meal was less than that after breakfast, but when the interval was four or more hours, the second rise was equal to or greater than the first one. It will be noted that the curves from the patients with mild diabetes have the same general features except that they are consistently on a somewhat higher level. In these especially is the higher after-breakfast portion of the curve interesting, as the sugar concentration often exceeded the threshold value for glycosuria only at that time. This is the justification for the common practice of giving insulin in these mild cases only before the first meal of the day. It is not, as sometimes supposed, because the insulin has an effect throughout the full day. In our more severe cases without insulin (Charts 10 and 11), the sugar concentration is seen to be high in the latter part of the day as well as in the morning, one curve keeping well above the threshold value for glycosuria throughout the day. These patients would necessarily, if kept on the same diets, require an insulin effect continuously.

Our other curves represent experiments with the administration of insulin to diabetic patients in varying stages of the disease. In the beginning (Charts 12, 13 and 14), we followed the Toronto plan of giving it before each meal, believing that it was not effective for more than four hours, on the average, and wishing to supply it at the times that food was being absorbed. It soon became apparent, however, that unnecessarily low blood sugar values in the afternoon were being obtained. One of us (L. J.[10]) showed this in a previous article. It is also to be seen in curves published by Wilder, Boothby, Barborka, Kitchen and Adams,[11] by Williams [12] and by others. Consequently, we learned that the midday dose of insulin could well be dispensed with. In all but the very mild cases, however, the dose before supper is clearly indicated, as many of our curves in which this dose was omitted will show (Charts 16, 17, 19, 22, 23 and 24). In certain others, even small doses of insulin in the late afternoon were not sufficient to keep down an evening rise. We also discovered that in many severe cases, on which unfortunately we were not able to procure satisfactory curves

10. Jonas, L.: A Report of Sixty-Four Cases of Diabetes Mellitus Treated with Insulin, Am. J. M. Sc. **166**:687-699 (Nov.) 1923.

11. Wilder, R. M.; Boothby, William; Barborka, C. M.; Kitchen, H. D., and Adams, S. F.: Clinical Observations on Insulin, J. Metabolic Res. **2**:701-728 (Nov.-Dec.) 1922.

12. Williams, J. R.: A Clinical Study of the Effects of Insulin in Severe Diabetes, J. Metabolic Res. **2**:729-751 (Nov.-Dec.) 1922.

for inclusion herein, large doses of insulin before supper did not prevent glycosuria and high blood sugar values in the early morning.

In an effort to overcome this high morning blood sugar, we experimented with a midnight dose of insulin, thus giving these patients three doses a day at approximately eight hour intervals (8 a. m., 5 p. m. and midnight). Knowing that recovery from the effects of the insulin given before supper did not occur until about that time, we did not feel that it was safe to give it earlier. The results from this timing of the insulin dosages have been commented on in connection with the detailed explanation of our curves, and it need only be noted here that Allen and Sherrill [13] have emphasized the need for an insulin effect continuously in such cases, no matter how the food is administered, in order to care for the glycogen which is constantly being broken down. They favor its administration before each meal and, in certain severe cases, at six hour intervals. Still more recently, Allen [14] has suggested that the last dose for the day be given an hour after supper or at bedtime. Our data, however, indicate the uselessness, and possibly even the harm from hypoglycemia, of a midday dose in the ordinary case. When two doses a day, given before breakfast and supper, respectively, do not rid a patient of glycosuria and the morning fasting level remains high, we unhesitatingly favor the extra dose being given late enough in the evening to reduce definitely the early morning value. This, we believe, is much more apt to occur if it is given within at least eight hours of the morning feeding, usually about midnight. Whether or not a small amount of carbohydrate should be given at the same time to overcome the possibility of hypoglycemia within the next hour or two depends on the patient's clinical reaction or on a blood sugar determination made one or two hours after the administration of the insulin. In some cases, we have found it necessary. On the other hand, we now have under observation a woman who has been receiving 15 units each midnight without any food, and at no time has she shown any evidence of hypoglycemia.

If we review all our curves, it will be noted that without insulin the highest blood sugar concentration usually occurs one hour after breakfast and with insulin, either at that same time or just before the administration of the first dose of insulin for the day. They also show that practically the lowest blood sugar level without insulin is to be found just before breakfast and that with insulin as we have used it, the lowest level is just before lunch or in the midafternoon. From this, it

13. Allen, F. M., and Sherrill, J. W.: Clinical Observations with Insulin, J. Metabolic Res. **2**:803-985 (Nov.-Dec.) 1922.

14. Allen, F. M.: Timing of Insulin Doses, J. A. M. A. **82**:1937 (June 14) 1924.

follows that the high and the low points in any case may be discovered by making in one day three routine determinations: one before breakfast or before the first insulin administration, if that is being used; one an hour after breakfast, and one just before lunch. By means of these three estimations, a reasonably accurate picture of the full day curve can be formed. The upper curve in Chart 32 was so obtained. For practical purposes, these are the only determinations of the blood sugar needed in the ordinary case, though in certain severe ones, it may be helpful to know, in addition, the level in the early evening, an hour after supper, by which the presupper dosage of insulin may be gaged, and the midnight concentration.

Another point of practical importance may be drawn from the fact that in diabetic patients without insulin, the highest blood sugar concentration develops soon after breakfast. If sugar occurs in the urine, it would be expected in greatest amount at the same time. We have repeatedly found this to be true, glycosuria often being present at only this time in the day. In consequence, when only a single specimen of urine can be obtained in suspected cases of diabetes, as often happens in office practice, one voided an hour or more after breakfast should be secured.

SUMMARY AND CONCLUSIONS

1. A series of thirty-four blood sugar concentration curves were made on twenty-nine patients, six nondiabetic and twenty-three diabetic, and covered periods up to 24 hours. This was done in an effort to work out a more satisfactory routine method of regulating the dosage and the time of administration of insulin to diabetic patients.

2. The ideal method was not arrived at, but the following general rules for the management of such cases seem warranted:

(*a*) With a maintenance diet equally distributed among the three meals of the day, mild cases of diabetes may be kept within the normal limits of glycemia by means of a single dose of insulin administered a half-hour before breakfast.

(*b*) More severe cases require, in addition to an adequate morning dose, a second but somewhat smaller one a half-hour before the evening meal.

(*c*) When the morning fasting level of blood sugar cannot be kept below the threshold value for glycosuria by these two doses, a third one is indicated at about midnight.

(*d*) The amount of insulin to be administered at each time must be determined by blood sugar estimations made before and after the meals.

3. When insulin is not being given and the diet factors are the same for each of the three regular meals, the highest blood sugar concentration occurs usually about one hour after breakfast, and the lowest is before breakfast.

4. When insulin is used, as suggested in the foregoing, the highest point may be attained before the first insulin administration of the day or an hour after breakfast, while the lowest point is usually reached just before the midday meal or in the midafternoon.

5. For practical purposes, it is suggested that, in the study of all diabetic patients, three blood sugar determinations should be made on one day; one on a specimen collected before the breakfast or before the first administration of insulin, if that is being used; one an hour after the breakfast, and a final one just before the luncheon. By these three estimations a reasonably accurate picture of the full day curve can be formed.

6. In all cases of diabetes, the single specimen of urine which is most likely to show sugar is one voided from one to two hours after breakfast.

PANCREATIC AND HEPATIC ACTIVITY IN DIABETES MELLITUS

THE ALTERATIONS WITH SOME OBSERVATIONS ON THE ETIOLOGY OF THE DISEASE *

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A brief preliminary report [1] has already been made of the findings obtained in a study of pancreatic and hepatic activity in cases of diabetes mellitus. It is the purpose of this paper to present these findings in detail and, so far as our findings seem to bear on them, to comment on certain considerations in this disease.

REVIEW OF THE LITERATURE

Although the recent work of Banting [2] and his collaborators has contributed an invaluable addition to our methods of treating diabetes mellitus, there still remains much to be desired in our knowledge of the actual etiology of this disease. That a deficiency of the internal secretion of the pancreas is the immediate cause of the glycosuria is incontestably proved, but the extent to which other organic functions are involved still remains a question. According to Allen,[3] in most, if not in all, cases of diabetes mellitus there must have been a preceding disease of the pancreas. This investigator believes that such pancreatic disease usually involves damage to both the insular and acinous cells. According to Joslin,[4] although proof for the cause of the disease is still lacking, "an antecedent pancreatitis would appear to be the most

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* From the Medical Services of the Massachusetts General Hospital, and the New England Deaconess Hospital.

This paper is No. 28 of a series of studies in metabolism from the Harvard Medical School and allied hospitals. The expenses of this investigation have been defrayed in part by a grant from the Proctor Fund of the Harvard Medical School for the Study of Chronic Disease.

1. Jones, C. M.: Alterations in Liver Function and in the External Secretory Activity of the Pancreas in Diabetes Mellitus: A Preliminary Report, Boston M. & S. J. **189**:851 (Nov. 29) 1923.

2. Banting, F. G., and Best, C. H.: The Internal Secretion of the Pancreas, J. Lab. & Clin. Med. **7**:251 (Feb.) 1922.

3. Allen, F. M.: The Pathology of Diabetes, J. Metabol. Res. **1**:165-251 (Feb.) 1922.

4. Joslin, E. P.: The Treatment of Diabetes Mellitus, Ed. 3, Philadelphia, Lea & Febiger, 1923, p. 137.

logical explanation". The typical pathology of the disease was first noted by Opie [5] in 1900, and later by Weichselbaum and Stangl,[6] who described the hydropic degeneration and vacuolization of the island cells. In studying the pathology of the pancreas in diabetes in human beings, Allen found few specimens of the pancreas which did not show marks of some damage or infection as the presumable cause of diabetes. These changes, as a rule, involved a quantitative loss in the number of islands, due to hyalinization and fibrosis. The effect of acute infections on the pancreas has been noted by Mallory [7] and consists chiefly in mitosis and regeneration of the cells in the islands, an indication of a preceding destruction or damage to these cells. In hemochromatosis or diabète bronzé we have a clear picture of pancreatic fibrosis and cellular degeneration, both insular and acinous, together with glycosuria.

In addition to the probability that pancreatic pathology is always an antecedent to the occurrence of diabetes mellitus, there exists further clinical and pathologic evidence that the primary or associated cause may be found in disease of the biliary tract. Moynihan [8] says, "chronic pancreatitis is generally due to gallstone irritation . . . The importance of the early recognition and treatment of chronic pancreatitis cannot be exaggerated, for the disease, if left unchecked, may produce such sclerosis of the gland that the whole secretory substance and the islands of Langerhans may be destroyed. The result is diabetes." Judd [9] believes that "cholecystitis is nearly always associated with a certain grade of hepatitis or pancreatitis, or both . . . Pancreatitis occurs frequently with cholecystitis and, as a result, a definite gross change occurs in the pancreas . . . it is possible for infection to invade the pancreas by way of the lymphatics from the gallbladder, and in many cases this probably explains the source of infection; it is apparently entirely relieved by treatment for cholecystitis." A comprehensive report by Lichty and Woods [10] on the significance of glycosuria in gallbladder disease contributes further suggestive evidence in the same direction. That hepatitis is usually associated with gallbladder

5. Opie, E. T.: Diabetes Mellitus Associated with Hyaline Degeneration of the Islands of Langerhans of the Pancreas, Bull. Johns Hopkins Hosp. **12**:263, 1901.

6. Weichselbaum, A., and Stangl, E.: Zur Kenntnis der feineren Veränderingen des Pankreas bei Diabetes Mellitus, Wien. klin. Wchnschr. **14**:968, 1901.

7. Mallory, F. B.: Quoted by Joslin, footnote 4.

8. Moynihan, B.: Abdominal Operations, Ed. 3, Philadelphia, W. B. Saunders Company, 1914, pp. 402, 403.

9. Judd, E. S.: Relation of the Liver and the Pancreas to Infection of the Gallbladder, J. A. M. A. **77**:197 (July 16) 1921.

10. Lichty, J. A., and Woods, J. O.: Significance of Glycosuria in Gallbladder and Duct Diseases, Am. J. M. Sc. **167**:1 (Jan. 1) 1924.

disease has been emphasized by Judd [9] and Peterman et al.,[11] and it is recognized that in catarrhal jaundice, a disease largely involving the liver, there may be an associated glycosuria. Of great interest in this connection is a very recent report by Frissell and Hajek.[12] These authors mention the development of glycosuria in three young girls following an attack of infectious jaundice. In two of the patients the glycosuria was apparently transient, but in the third case a full-blown and rather severe case of diabetes mellitus resulted.

That there is a close relationship between the liver and pancreas in carbohydrate metabolism is unquestioned. Various theories have been propounded attempting to explain the rôles played by these organs in the utilization of glucose, but until the isolation and use of insulin little concrete evidence had been obtained as to the actual processes concerned. A recent review of the subject by MacLeod [13] indicates clearly two facts. In diabetic animals the administration of insulin enables the liver to store glycogen, and is associated with a rise in the respiratory quotient. The exact significance of the latter is not entirely clear, although it undoubtedly indicates an increased utilization of glucose. Although numerous investigators have made extensive studies of the subject, there is still a question as to the exact mechanism by which glucose is utilized. Whether the existing glucose is changed into an active form, the so-called gamma glucose of Winter and Smith,[14] or whether the tissues are enabled to oxidize more readily glucose, or the liver to supply it in an available form, is still unknown. According to Geelmuyden [15] and other German investigators, insulin acts by inhibiting the overproduction, by the liver and other tissues, of glucose from fat, a distinctly novel theory. Whatever the precise mechanism involved may be, it is evident that in diabetes mellitus the diminution or absence of the internal secretion is the primary consideration, but it is also probable that in addition there is a marked disturbance in hepatic function, which is associated with lack of glycogen formation and storage. Pathologically, it is well recognized that diabetic livers are practically depleted of their glycogen deposits. In this connection the striking work of Mann and Magath [16] is of extreme interest. These

11. Peterman, M. G.; Priest, W. S., and Graham, E. A.: The Association of Hepatitis with Experimental Cholecystitis and Its Bearing on the Pathogenesis of Cholecystitis in the Human, Arch. Surg. **2**:92 (Jan.) 1921.

12. Frissell, L. F., and Hajek, Joseph: Insulin in the Severer Forms of Diabetes, with Report of Cases, Arch. Int. Med. **33**:230 (Feb.) 1924.

13. MacLeod, J. J. R.: Insulin, Physiol. Rev. **4**:21 (Jan.) 1924.

14. Winter, L. B., and Smith, W.: Nature of Sugar in the Blood, J. Physiol. **57**:100 (Dec.) 1922.

15. Geelmuyden, H. C.: Metabolism in Diabetes, Klin. Wchnschr. **2**:1677 (Sept. 3) 1923.

16. Mann, F. C., and Magath, T. B.: Studies in the Physiology of the Liver, Arch. Int. Med. **30**:73 (July), 171 (Aug.) 1922; ibid. **31**:797 (June) 1923.

investigators, in animal experiments, conclusively showed that the liver is of great importance in the normal control of carbohydrate metabolism, and that there exists an intimate relation between the liver and the pancreas in this process. In their animals hepatectomy produced death due to hypoglycemia, on account of the removal of the chief agent for storage and production of available glucose. The hyperglycemia following pancreatectomy was found to depend in a large measure on the presence of the liver, since removal of that organ after pancreatectomy caused an immediate drop to an abnormally low level in the previously high blood sugar.

In this connection the work of Ohler and Broun [17] is of interest. These investigators, in work as yet unpublished, have attempted to produce evidence of a disturbance of liver function in diabetes mellitus. The recently introduced dye test for hepatic function, phenoltetrachlorphthalein test, was employed in studying a series of diabetic patients. In no case without obvious liver pathology was there any abnormal retention of the dye in the blood serum. Although such results undoubtedly indicate the absence of profound liver changes, it is to be questioned whether they present any evidence against the existence of a functional disturbance of the liver in diabetes mellitus. It is also of interest to mention the fact that these observers found that cases of liver disease of sufficient severity to show a retention of phenoltetrachlorphthalein in the blood stream also showed a reduced sugar tolerance. It may be added that present evidence would seem to indicate that the dye test for liver function, although an improvement on many older methods, still leaves much to be desired as a method for detecting any but gross disturbances of liver function.

That the internal secretion of the island cells of the pancreas and the integrity of the hepatic function are not the only factors affected in diabetes mellitus, and that insulin alone may not be entirely sufficient in controlling all the features of the disease, is suggested by recent investigative reports. Allen [3] is responsible for the statement that the differences that still exist between a "total" diabetic animal and a totally pancreatectomized animal "furnish evidence, first, that the profound cachexia following total pancreatectomy is not due solely to the failure of carbohydrate metabolism or the hyperglycemia or glycosuria resulting from this failure; and second, that the alpha duct, acinous or other cells of the pancreas furnish an unknown internal secretion, which is somehow important for the welfare of the organism." Somewhat similar conclusions have been reached by Fisher,[18] who attempted

17. Ohler, W. R., and Broun, G. O.: Boston City Hospital, personal communication.

18. Fisher, N. F.: Attempts to Maintain Life of Totally Pancreatectomized Dogs Indefinitely by Insulin, Am. J. Physiol. **67**:634 (Feb.) 1924.

to keep alive pancreatectomized dogs by the use of insulin. Only those dogs in which there was some apparent regeneration of the duodenal stump of the pancreatic duct were observed to remain alive for long periods of time. He concludes that insulin probably does not represent the entire pancreas hormone complex, since in the total absence of the pancreas insulin cannot maintain life or does not control all the diabetic symptoms (subnormal weight, polyphagia, polyuria).

In view of the foregoing experimental and clinical data it is evident that diabetes mellitus, in addition to the known diminution of the internal secretion of the island cells of the pancreas, may well exhibit abnormalities in liver function and in the external secretory activity of the pancreas. It is with the hope of providing acceptable evidence of such a derangement of the normal physiologic processes that the following observations are presented. The studies consist in estimations of the pancreatic enzyme activity and the bile pigment concentration of the duodenal contents in sixty-eight cases of diabetes mellitus.

CLINICAL MATERIAL

The cases under consideration consist of an unselected group of sixty-eight cases of diabetes mellitus, taken from the wards of the Massachusetts General Hospital and the New England Deaconess Hospital. The youngest patient was aged 11 years, the oldest aged 72. The average age was 44 years. Of the sixty-eight patients, about 80 per cent. were over 20, and nearly half of the patients were aged 50 or over. The age distribution in these cases is fairly representative of the disease, although there is probably a slight preponderance of cases in adult life. The distribution by sexes was essentially that accepted in most of the statistical data, i. e., females 55 per cent., males 45 per cent. The average known duration of the disease was about two and three-quarters years. In a few patients the disease had apparently existed for only one or two months, and in several cases symptoms had been present for well over ten years.

Although the majority of the patients entered the hospital for dietary regulation, twenty-eight of them were in a state of mild or severe acidosis on admission. Twelve patients were examined while definitely acidotic. One patient entered the hospital in a deep coma from which he recovered, following the administration of insulin. Thirteen exhibited, on admission, infection or gangrene. There were three cases of hemochromatosis, and one of cancer of the body and tail of the pancreas. One patient had a known history of acute pancreatitis requiring surgical intervention, a second one was known to have chronic pancreatitis following a long period of gallbladder attacks, and two others were probable cases of chronic pancreatitis. Thirty-one patients showed marked arteriosclerosis, a finding entirely consistent with the

age distribution of the cases. Regarding the incidence of gallbladder disease more will be said in the discussion of the laboratory findings.

The foregoing group of patients constitutes a fair average group of hospital diabetic patients, with the possible exception of the three cases of hemochromatosis. In spite of the number of patients showing more or less evidence of acidosis, the average severity of the group is but little higher than that of any similar number of patients with the disease.

In addition to the before mentioned patients, a group of ten normal persons was studied for normal control figures.

METHOD

The methods employed in the examination of these patients have been described in articles already published, and are, briefly, as follows: The patients were invariably examined in a state of fasting. A duodenal tube was passed into the duodenum and the position of the tip verified by fluoroscopic examination. As soon as the tip of the tube was in the middle of the second portion of the duodenum, 50 c.c. of a warm 33 per cent. solution of magnesium sulphate was instilled into the duodenum. Shortly thereafter a free flow of bile was obtained, due to the relaxation of the sphincter of Oddi, and collection of the contents was made for the next half hour. At the end of this period the patient was given a test meal of 40 c.c. of 40 per cent. cream by mouth. As soon as the cream was observed in the duodenal contents, a separate collection was made for a period lasting one hour.

The first portion of the duodenal contents was examined as soon as collected for bile pigments and sediment. The second portion, collected after the cream meal, was examined for pancreatic enzyme activity.

The method of examining the duodenal contents for bile pigments has been described in a recent article [19] and is here quoted.

> To 10 c.c. of duodenal contents add an equal amount of a saturated alcoholic solution of zinc acetate. This is shaken and filtered, and to 10 c.c. of the filtrate is added 1 c.c. of Erlich's reagent. This solution is allowed to stand in the dark for fifteen minutes and is then examined spectroscopically for the presence of bilirubin, urobilin, and urobilinogen. The number of dilutions with 95 per cent. alcohol necessary to remove the characteristic absorption band of a given pigment is taken as the relative amount of that pigment in a given specimen. The absorption bands of urobilin and urobilinogen are absolutely characteristic. Although it has been said that bilirubin gives no characteristic shadow in the spectrum, I have found that pure solutions of this pigment give a distinctly characteristic shadow in the blue-violet end of the spectrum. As the concentration of this pigment increases, the shadow involves the blue-green, and even the yellow-green. In such instances the shadow includes the band made by the urobilin present and renders it impossible to estimate the latter pigment. In this event the original sample, after being treated with zinc acetate and Erlich's reagent, is split in two portions. One specimen is examined for the presence of bilirubin and urobilinogen, and

the dilutions estimated. The second fraction is treated with an equal amount of a 10 per cent. solution of calcium chlorid. The addition of the calcium precipitates sufficient bilirubin to permit the estimation of the urobilin present. In normal persons the normal concentrations have been found to be: bilirubin, from 60 to 150 dilutions; urobilin, 4 to 16 dilutions; urobilinogen, from 0 to 4 dilutions.

At the time when this investigation was commenced attempts to estimate the urobilin content were unsatisfactory, and as a result some of the cases included in the tables have no figure for urobilin.

Estimation of the sediment was also performed by the method outlined in the above article. It consists in high speed centrifugalization of specimens of the duodenal contents for fifteen minutes, and subsequent miscroscopic examination of the sediment thus obtained. The observations made by Jones [19] indicate clearly that the finding of abnormal amounts of cholesterin, bilirubin or calcium bilirubin crystals in the duodenal sediment constitutes strong evidence for a diagnosis of cholelithiasis. These observations were made on a large number of cases, and the conclusions seem to have been entirely justified by further clinical experiences. The findings of large amounts of bile-stained cells, epithelium or leukocytes has been found to be highly suggestive of biliary tract inflammation, but is not diagnostic of any given disease entity. Other microscopic findings are probably of no significance, and the examination of the sediments in this group of cases was entirely confined to a search for the above-named elements.

The method used for the determination of enzymatic activity was originally described by McClure, Wetmore and Reynolds,[20] and was subsequently employed by McClure and Jones [21] in a clinical study of cases of pancreatic disease. By means of this method, the latter were able to demonstrate a marked diminution of proteolytic, lipolytic, or amylolytic activity below previously determined minimum normal limits in patients with known pancreatic pathology. The technic briefly described is as follows:

Proteolytic activity is estimated by allowing a dilution of the duodenal contents to act on a solution of soluble casein. The casein not affected by proteolytic action is precipitated by means of metaphosphoric acid solution. The index of proteolytic activity is taken as the number of milligrams of nitrogen not precipitated by the metaphosphoric acid. This nitrogen value is determined by an adaptation of the method of Folin and Wu for the determination of nonprotein nitrogen in the blood. Amylolytic activity is estimated

19. Jones, C. M.: The Rational Use of Duodenal Drainage. An Attempt to Establish a Conservative Estimate of the Value of This Procedure in the Diagnosis of Biliary Tract Pathology, Arch. Int. Med. **34**:60 (July) 1924.

20. McClure, C. W.; Wetmore, A. S., and Reynolds, Lawrence: New Methods for Estimating Enzymatic Activities of Duodenal Contents of Normal Man, Arch. Int. Med. **27**:706 (June) 1921.

21. McClure, C. W., and Jones, C. M.: Studies in Pancreatic Function, Boston M. & S. J. **187**:909 (Dec. 21) 1922.

as the number of milligrams of glucose developed by action of duodenal contents on a solution of soluble starch. The index of amylolytic activity is taken as the total number of milligrams of glucose developed, as determined by the method of Folin and Wu for the determination of sugar in the blood. Lipolytic activity is estimated by allowing duodenal contents to act on a true emulsion of cottonseed oil, and determining the amount of acidity developed by titrating with tenth normal alcoholic solution of sodium hydroxid. The number of cubic centimeters of tenth normal sodium hydroxid necessary to neutralize the acidity developed is used as the index of lipolytic activity. The samples of duodenal contents and the reagents used must be controlled for the presence of nitrogen not precipitated by metaphosphoric acid, for copper reducing bodies and for acidity.

The figures established by McClure and his associates as the minimal values for enzymatic activity were as follows: proteolytic, 2.0 mg. nonprotein nitrogen; lipolytic, 1 c.c. of tenth normal sodium hydroxid; amylolytic, 1 mg. glucose. As may be seen in Table 1, the

TABLE 1.—*Comparison of Duodenal Findings in Normal Controls and Diabetic Patients*

	Pancreatic Enzyme Activity			Duodenal Bile Pigments			
	Proteolytic, Mg. Nonprotein Nitrogen	Lipolytic, C.c. N/10 Sodium Hydroxid	Amylolytic, Mg. Glucose	Urobilinogen Dilution Units	Urobilin Dilution Units	Bilirubin Dilution Units	Sediment
Standard values	1.5	0.75	0.75	4	16	150	0
Average findings in 10 normal persons	2.8	1.6	2.2	0	12	120	0
Average findings in 68 diabetic patients	1.9	1.0	2.5 (57 cases)	10	25 (41 cases)	240	11
Average findings in cases showing abnormalities	1.60	0.40	0.40	24	44	313	—
Per cent. abnormalities in 68 diabetic patients	33%	37%	9%	40%	50%	67%	19%

average results obtained in studying our group of normal controls were all well above McClure's minimal values. On rare occasions, however, we have obtained in normal persons, isolated figures slightly below these values. In order to obtain results that will leave little room for criticism we have arbitrarily chosen for minimal values figures which are but 75 per cent. of McClure's lowest normal figures. Thus, we have assumed that values below the following may be safely construed as showing evidence of diminished pancreatic enzymatic activity: proteolytic, 1.5 mg. nonprotein nitrogen; lipolytic, 0.75 c.c. tenth normal sodium hydroxid; amylolytic, 0.75 mg. glucose.

INVESTIGATIVE FINDINGS

Examination of Table 1 reveals several facts of interest. The average findings in the determination both for activity and bile pigment elimination in the group of normal controls were all well within normal limits.

The enzyme estimations were far above even the minimal values given by McClure, and the bile pigment estimations were below the upper limits of normal. An average of the various findings on the group of sixty-eight diabetic patients shows that the estimation for proteolytic and lipolytic activity was distinctly below the average figures for the normal controls, but were above our minimal normal limits. Figures for amylolytic activity were above the normal control averages, and very much higher than our minimal values. The average values for the individual bile pigments, however, were all far in excess of control figures, and the normal standard limits.

In spite of the fact that the average figures for enzymatic activity in diabetic patients do not show a diminution below our arbitrarily adopted minimal standards, it is to be noted that the protein and fat splitting enzyme average values were just at the lower levels established by McClure. Turning from average figures to individual results, however, we find that 33 per cent. of the cases showed proteolytic activity below our figure of 1.5 mg. nonprotein nitrogen, and 37 per cent. were below our minimum figure of 0.75 c.c. tenth normal sodium hydroxid for lipolytic activity. In the case of the starch-splitting enzyme, only 9 per cent. of the cases developed values below 0.75 mg. glucose.

From the bile pigment estimations it was found that 40 per cent. of the cases showed abnormally high values of urobilinogen, 56 per cent. for urobilin, and 67 per cent. gave high values for bilirubin.

Of the entire sixty-eight cases, thirty-three, or 49 per cent. showed a diminution in the activity of one or more enzymes, and fifty-one cases, or 76 per cent., showed an abnormal output of one or more of the bile pigments. Twenty-three cases, or one-third of the total number studied, showed abnormalities both in the enzymatic activity and bile pigment elimination.

Examination of the last line of data in Table 1 reveals the average level of enzyme and bile pigment estimations in those cases showing any abnormality. The general average of this group of cases is not only different from that of the normal controls, but is strikingly abnormal in relation to our minimal enzyme and maximum pigment limits.

Studies of the duodenal sediments showed eleven cases, or 19 per cent., in which there was a large amount of cholesterin or bile pigment crystals. Four more cases showed large amounts of bile-stained cells, both epithelial and leukocytic.

In order to attempt an analysis of the significance of the foregoing findings a tabulation has been made of the usual variables in diabetic patients, and the percentage of abnormal findings under these varying

conditions has been outlined. Table 2 indicates the effect of the most common variables in diabetes mellitus, namely, sex, age, duration of the disease, the fasting blood-sugar level at the time of examination, the diet in calories per kilogram of body weight, and the administration of insulin, both as regards dosage and duration of treatment.

TABLE 2.—*Effect of Change in Usual Diabetic Variables*

Diabetic Variables	Number Cases	Percentage of Cases with Abnormal Enzyme Activity	Percentage of Cases with Abnormal Bile Pigments
Average findings on 68 diabetic patients	68	49	76
Sex:			
Male	31	47	80
Female	37	57	73
Age:			
Under 30 years	13	69	69
30 to 50 years	21	48	72
Over 50 years	34	55	85
Duration of Diabetes:			
Under 1 year	20	53	84
1 to 3 years	22	41	68
Over 3 years	26	65	73
Fasting Blood Sugar:			
Under 0.15 per cent.	27	42	80
0.15 to 0.20 per cent.	16	56	56
Over 0.20 per cent.	22	59	86
Diet, Calories per Kg. of Body Weight:			
Under 10 calories	8	50	100
10 to 20 calories	26	56	80
20 to 30 calories	18	33	72
Over 30 calories	15	66	66
Insulin Therapy:			
No insulin	38	49	73
Under 10 units per day	10	30	80
10 to 20 units per day	11	55	90
Over 20 units per day	9	89	66
Insulin Therapy:			
No insulin	38	49	73
4 days or under	13	30	70
Over 4 days	17	53	76

TABLE 3.—*Effect of Complications on Diabetes*

Complications	Number Cases	Pancreatic Enzyme Activity, per Cent. Abnormal	Duodenal Bile Pigments, per Cent. Abnormal
Average determinations on 68 cases	68	49	76
Acidosis within 2 weeks	15	41	94
Acidosis at examination	12	50	75
Sepsis, gangrene	10	50	90
Marked arteriosclerosis	31	50	77
Cholelithiasis (probable)	15	53	60
Pancreatitis (probable)	4	100	100
Hemochromatosis	3	100	0

In Table 3 we have attempted to outline the effect of complicating factors on the pancreatic and hepatic activity. Acidosis, sepsis, gangrene, arteriosclerosis, gallbladder disease, pancreatitis, and hemochromatosis are tabulated in order named, with the apparent relation of each factor to the percentage of abnormal findings.

Table 4 gives in detail the average findings in five cases which were examined more than once. In each case the initial examination was made while the patient was in a state of severe acidosis. A second observation was made at a subsequent date when the patient had apparently recovered from the acidosis. From the values in this table, it is obvious that in these patients recovery from acidosis was associated with a marked change in pancreatic and hepatic function.

In Table 5 all of the important data on each individual diabetic patient are given in full detail. The findings include sex, age, duration of the disease, urinary and blood sugar at the time of examination, the presence or absence of urinary diacetic acid, weight in kilograms, dietary intake in calories per kilogram of body weight, insulin dosage, the enzymatic and bile pigment estimations, sediment findings, and any other data of importance. Examination of this table shows that, in

TABLE 4.—*Changes Occurring After Recovery from Severe Acidosis*

	Pancreatic Enzyme Activity			Duodenal Bile Pigments		
	Proteolytic	Lipolytic	Amylolytic	Urobilinogen	Urobilin	Bilirubin
Standard values	1.5	0.75	0.75	4	16	150
Average findings in 5 patients during acidosis	1.36	0.61	2.61	16	46	281
Average findings in same 5 patients after recovery	1.88	1.12	1.98	1.2	26	202
Percentage apparent improvement in function	+38%	+83%	−24%	+920%	+43%	+28%

spite of the average level of enzymatic activity being above the minimum normal figures, a large number of cases showed striking reductions in enzyme action. In six cases (Cases 9, 17, 23, 34, 39, 41) one or more of the enzymes showed almost negligible findings, and in one case (Case 56) it was impossible to produce any evidence of enzymatic activity. In the last named case, identical findings were obtained at a subsequent examination one year later. It is obvious from Table 1 that those cases showing abnormal findings gave average values of from 30 to 45 per cent. below our minimal normal figures, and over 50 per cent. below McClure's minimum values. Similarly, in the bile pigment estimations, those cases showing abnormalities gave average findings of 150 per cent. or more above the upper limits of normal. In the face of such findings, therefore, there can be little doubt that real abnormalities exist both in pancreatic enzyme activity and in bile pigment metabolism in a large percentage of cases of diabetes mellitus. These abnormalities consist in a diminution of the external secretory activity of the pancreas, and in an increase in the bile pigment metabolism.

TABLE 5.—*Summary of Data on Cases at Time of Intubation*

Case	Sex*	Age	Duration in Years	Fasting Blood Sugar, per Cent.	Urinary Sugar, per Cent.	Diacetic Acid	Weight, Kg.	Calories per Kg. Body Weight	Units Insulin per Day	Duodenal Findings: Enzymatic Activity: Proteolytic, Mg. Nonprotein Nitrogen	Duodenal Findings: Enzymatic Activity: Lipolytic, Sodium Hydroxid. C.c. N/10	Duodenal Findings: Enzymatic Activity: Amylolytic, Mg. Glucose	Duodenal Findings: Bile Pigments: Urobilinogen Dilution Units	Duodenal Findings: Bile Pigments: Urobilin Dilution Units	Duodenal Findings: Bile Pigments: Bilirubin Dilution Units	Sediment	Remarks
1	♀	14	1.0	0.16	0	+	38	32	30	1.4	1.4	3.6	0	4	64	0	Entered with acidosis
2	♀	47	4.0	0.24	0	0	86	5.6	0	4.0	1.9	6.8	6	24	370	+	Cholelithiasis (?)
3	♀	15	0.1	0.10	0	0	34	40	30	1.1	1.1	1.7	0	8	162	0	Entered with acidosis
4	♀	48	3.0	0.13	0	0	85	12	0	0.6	0.45	1.2	0	4	48	+	Cholelithiasis (?); sepsis
5	♀	27	0.5	0.11	0	+	63	20	45	0.7	0.3	0.2	6	22	130	0	Entered with acidosis; acute pancreatitis (?)
6	♂	58	2.0	0.64	0	0	56	28	0	1.7	0.9	3.1	64	140	820	0	Gangrene; entered with acidosis
7	♀	50	7.0	0.16	0	0	47	33	30	2.5	1.7	2.3	3	16	266	0	
8	♂	63	2.0	0.18	+	0	87	15	0	2.5	1.6	2.6	4	8	180	0	Entered with acidosis; sepsis
9	♂	11	1.0	0.15	0.9	0	22	43	60	0.9	0.2	1.5	0	6	36	0	Entered with acidosis
10	♂	51	0.6	0.17	0	0	60	25	7	2.7	1.8	3.6	0	12	130	0	Syphilis
11	♂	30	4.0	0.20	0	0	50	17	20	1.4	0.5	2.5	2	40	216	0	Entered with acidosis
12	♀	17	1.0	0.21	0.8	+	40	5	0	2.3	1.6	2.9	0	72	324	0	Entered with acidosis; sepsis three weeks before
13	♂	44	3.0	0.13	0	0	53	15	16	1.8	1.1	2.5	35	150	342	0	Entered with acidosis
14	♀	53	13.0	0.29	+	0	60	11	6	3.0	1.1	0.5	4	108	216	0	Syphilis
15	♂	31	2.5	0.20	0	+	63	22	14	1.1	0.7	1.2	3	45	200	+	Cholelithiasis (?); entered with acidosis
16	♂	61	1.3	0.15	0	0	54	15	0	1.5	1.0	2.2	2	16	620	0	
17	♀	58	2.0	0.17	0	0	55	24	0	+	0.05	0.2	0	6	57	+	Cholelithiasis (?)
18	♀	56	2.0	0.28	+	0	60	12	6	2.1	1.3	3.3	4	22	288	+	Cholelithiasis at operation
19	♀	34	0.6	0.22	0	0	66	15	15	2.8	1.3	3.1	0	7	76	0	Sepsis
20	♂	23	1.0	0.38	0.3	0	53	12	0	2.6	0.9	3.2	0	18	132	0	
21	♀	72	9.0	0.13	0	0	75	19	0	1.6	0.8	3.6	0	16	104	+	Cholelithiasis
22	♀	45	8.0	0.28	0	0	80	8.7	9	2.8	1.3	4.6	12	28	216	0	Diagnosis: cholelithiasis in 1906
23	♀	50	1.0	0.18	1.8	0	33	40	15	1.5	0.1	3.0	12	36	162	0	Sepsis
24	♀	38	8.0	0.14	0	0	80	14	0	1.2	0.5	1.4	6	37	270	0	Gangrene
25	♂	55	0.3	0.26	4.8	+	82	12	6	3.0	1.6	4.6	0	33	258	0	Entered with acidosis
26	♀	45	1.7	0.08	0	0	53	30	0	2.6	1.4	2.9	0	16	258	0	
27	♂	49	0.1	0.08	0	0	84	21	0	1.8	1.5	2.6	0	12	259	0	Entered with acidosis
28	♂	65	7.0	0.20	2.0	++	56	14	15	1.8	0.7	2.6	0	18	350	0	Duodenal ulcer

29	♀	62	13.0	0.15	0	0	45	31	8	1.5	0.9	2.1	2	6	144	0	
30	♀	52	18.0	0.21	0.2	0	49	14	6	2.6	1.1	3.5	0	24	324	0	Entered with acidosis; cholelithiasis operation one year ago
31	♂	47	3.0	0.26	0.2	0	55	23	30	1.5	0.7	2.7	16	32	432	+	Cholelithiasis (?); entered with acidosis
32	♀	50	1.0	0.25	0	0	52	38	42	1.9	0.4	3.0	2	10	244	+	Cholelithiasis (?)
33	♂	32	0.6	0.09	0	0	50	24	5	2.4	1.6	2.7	27	40	421	0	
34	♀	39	0.5	0.21	0.2	0	54	10	15	3.3	0.2	3.4	0	12	324	0	Entered with acidosis
35	♀	56	9.0	0.23	0	0	69	22	15	2.1	2.0	3.4	0	12	300	0	Pancreatitis (?)
36	♂	61	6.0	0.11	0	0	58	27	0	2.8	1.1	3.1	0	10	102	0	Cholelithiasis (?)
37	♀	56	0.8	0.22	0.5	0	60	27	18	1.3	0.7	3.5	0	14	244	0	Entered with acidosis
38	♂	46	2.0	0.11	0	0	68	23	0	2.0	1.1	4.0	0	6	72	0	
39	♀	51	5.0	0.30	8.0	0	79	4	12	2.0	0.01	3.8	6	16	432	+	Cholelithiasis
40	♀	15	1.6	0.35	0.4	0	36	40	22	1.9	0.6	2.8	0	12	108	0	Pancreatitis (?)
41	♀	46	11.5	0.13	0	0	35	52	0	0.0	0.4	1.4	0	12	90	0	
42	♀	67	10.0	0.15	0	+	56	12	0	1.5	0.9	1.8	0	—	115	0	Entered with acidosis
43	♂	27	0.1	0.10	0	0	64	37	0	1.1	0.9	1.7	5	—	440	0	
44	♂	25	1.5	0.28	0.8	+++	54	25	10	1.6	2.3	1.0	4	—	260	6	Infection
45	♂	54	4.0	0.17	0	0	68	7	0	2.4	0.5	1.5	16	—	500	0	
46	♀	13	0.3	0.15	0	0	36	44	15	1.8	1.5	3.7	0	—	240	0	Entered with acidosis
47	♂	63	9.0	0.14	0	0	51	25	0	1.6	0.3	2.2	0	—	96	0	Hemochromatosis
48	♂	55	0.6	0.36	0	+	66	14	0	1.0	0.3	1.8	0	—	100	0	Hemochromatosis; entered with acidosis
49	♂	53	2.5	0.11	0	+	71	18	0	1.6	0.9	3.7	200	—	200	0	Entered with acidosis; infection
50	♀	39	0.3	0.13	+	0	50	29	5	1.9	1.0	2.2	4	—	200	0	Entered with acidosis
51	♀	28	2.5	0.25	1.0	+++	61	3	0	1.4	1.4	2.8	14	—	300	0	Entered with acidosis
52	♀	64	0.5	?	0	0	79	13	0	4.0	0.9	0.3	6	—	700	0	Cholelithiasis (?)
53	♀	55	4.0	?	+	0	69	?	0	1.5	0.3	1.9	2	—	90	0	Previous operation; cholelithiasis
54	♂	43	2.0	0.11	0	0	55	17	0	1.6	0.4	0.5	0	—	30	0	Hemochromatosis
55	♂	58	0.3	0.14	0	0	60	0	0	0.8	1.8	1.7	15	—	450	0	Cholelithiasis; pancreatitis
56	♂	54	0.5	0.22	3.4	0	67	11	0	0.0	0.0	0.0	2	—	220	0	Acute pancreatitis seven years ago
57	♂	48	0.5	0.10	0	0	47	17	0	2.5	1.6	2.0	9	—	350	0	Cancer of pancreas
58	♂	42	10.0	0.15	0	0	60	30	5	1.7	0.4	0.4	9	—	175	0	Infection; entered with acidosis
59	♂	57	9.0	0.11	0	0	69	28	0	2.1	1.4	0.6	4	—	100	0	Entered with acidosis
60	♂	65	2.0	0.10	0	0	46	47	0	2.9	1.4	1.3	0	—	330	0	
61	♀	43	1.5	0.13	0	0	75	18	0	2.6	2.1	1.3	3	—	400	0	
62	♀	57	6.0	0.13	0	0	90	18	0	1.7	0.4	0.4	0	—	160	0	Gangrene
63	♀	45	1.5	0.16	0	0	57	28	0	2.7	1.5	1.2	0	—	94	+	Cholelithiasis
64	♂	22	1.5	0.15	0	0	54	23	0	2.5	2.0	1.9	15	—	350	0	Entered with acidosis
65	♂	14	0.1	0.10	0	0	40	47	0	2.0	0.8	0.9	0	—	270	0	Entered in coma
66	♀	49	3.0	0.10	0	++	56	9	0	4.3	1.6	0.9	27	—	90	+	Entered with acidosis; cholelithiasis
67	♀	60	20.0	0.17	0	0	74	13	0	2.1	1.1	0.9	55	—	140	0	
68	♀	65	0.5	?	?	0	71	11	0	2.8	1.1	0.2	80	—	160	0	

* In this column, ♀ indicates female, ♂ male.

COMMENT

In view of the before mentioned findings it is of interest to discuss the probable significance of these alterations in pancreatic and hepatic function, and to comment briefly on the various factors concerned in their production. The first fact of importance is the diminution in the enzymatic activity of the duodenal contents. As already noted, nearly one-half of the cases showed a diminution in the activity of one or more of the pancreatic enzymes. The striking fact in these findings is that amylolytic activity was apparently diminished in only 9 per cent. of the cases, whereas proteolytic activity and lipolytic activity showed definite reductions below the minimum normal level in over a third of the cases. Just why there should be a dissociation of these ferment actions is extremely interesting, and at present we can offer no reasonable explanation of this fact. The changes described, however, can apparently have but one significance, namely, that the duct or acinous cells of the pancreas are severely damaged in a large proportion of cases of diabetes mellitus. Such a disturbance may be purely functional, or, as is more likely, may be anatomic.

To what extent the reduction in pancreatic enzyme activity noted in our cases may influence the symptomatology and course of the disease it is difficult to surmise. It is certain that in most cases the effect of this diminution of digestive activity can be at least partially compensated for by the presence of other ferments in the digestive tract. It is also certain that even striking abnormalities in the external secretion of the pancreas may be present without marked symptomatology, and not infrequently cases of cancer of the pancreas, in which the major part of the gland is entirely replaced by neoplastic tissue, are encountered without other symptoms than an unexplained weakness and some loss of weight. The observations of Allen and Fisher, referred to in the foregoing, would seem to indicate, however, that in pancreatectomized animals an additional factor to the loss of the internal secretion of the organ may contribute definitely to the symptoms of the disease. In Fisher's experiments the loss of weight, polyphagia and polyuria were inadequately relieved by insulin therapy alone and the animals did not survive. It may well be, therefore, that a marked diminution in proteolytic and lipolytic activity in cases of diabetes mellitus may contribute definitely to the symptomatology of the disease. Such symptoms, are, however, of relatively minor importance in the majority of cases, and are at present impossible to define. It is also improbable that in most cases the degree of disturbance of the external secretory activity of the pancreas, exhibited in our findings, exerts a very definite influence on the prognosis of the disease except where the actual degree of pancreatic damage is extreme. In

cases of chronic pancreatitis following acute pancreatitis, the usual resulting diabetes is generally severe and of short duration. Hemachromatosis, a disease in which the damage to the pancreatic tissue is widespread, is also usually marked by severe diabetes. With the exception of such cases, however, it would seem that disturbances of the external secretion of the pancreas exert little evident effect on the duration or severity of the disease. A practical point, however, may be noted here regarding the results of long continued diminution in the digestive activity of the pancreatic secretion. If such diminution is of sufficient degree there very well may be an associated disturbance in the actual absorption of food products. Diminished proteolytic and lipolytic activity might well be reflected in a diminished absorption of protein and fat, with a resulting increase in the protein and fat content of the stools. Such a theoretical consideration may possibly help to explain the apparent inability of certain diabetic patients to progress satisfactorily on a given diet, owing to the fact that a certain proportion of the food intake is lost in the stools. A further consideration is suggested by the fact that the largest percentage of abnormalities was noted in the determinations of lipolytic activity. Inability to split fat properly may explain the intestinal symptoms that are not too infrequently met with in patients with severe diabetes. Intermittent diarrhea is not uncommon in severe cases and during these attacks free fat and fatty acids appear in the stools in abnormal amounts. Although such intestinal disturbances are usually attributed to asthenia, plus an irritating diet of fruit or coarse vegetables, it is an interesting assumption to consider such phenomena as primarily due to an inability to split and absorb fat completely. Case 20 is a case in point. This patient was a woman, aged 59, with rather severe diabetes of one year's duration. Diarrhea was an outstanding symptom, and it is to be noted that the activity of the pancreatic lipase was reduced to almost a negligible quantity.

It is evident from a close examination of Table 5 that the severity of the diabetes does not necessarily run parallel to the degree of disturbance of the external pancreatic secretion. A striking example of this is seen in Case 41 from the New England Deaconess Hospital. This patient, a woman, aged 46, was first diagnosed as a diabetic patient about eleven years before the present examination. During those years she had been seen repeatedly by competent physicians, and had repeatedly shown hyperglycemia and glycosuria. At the time of our examination she was very much emaciated and weighed only 35 kg. (78 pounds). She was consuming a diet that afforded her 52 calories per kilogram of body weight, was sugar free, and had a normal blood sugar, without the use of insulin. In spite of this remarkable food intake she showed

no demonstrable proteolytic activity, and only a small amount of lipolytic activity. Amylolytic activity was within normal limits. With some few exceptions, of which the foregoing is an interesting example, those that were clinically mild diabetic patients tended to show little or no reduction in the enzymatic activity of the pancreas. The question naturally arises in a discussion of the cause of the reduction in enzyme activity, whether such a reduction may not be due primarily to emaciation alone. It is well known that marked undernutrition, with the resulting loss of weight, is accompanied by changes in the size and function of the pancreas. Loss of more than 30 per cent. of the body weight is said to be associated with demonstrable loss in pancreatic function. In such patients as Case 46, before mentioned, it is entirely possible that undernutrition played a part in the reduction of pancreatic enzyme activity. The majority of the patients included in this series, however, were not extremely emaciated, and the lowest figures for enzyme activity were not necessarily found in patients who were strikingly under weight. We have frequently found high values for enzyme activity in patients who were very emaciated, both in this series, and in examinations made on patients suffering from other diseases. It can be said, therefore, that undernutrition is an important factor in the reduction of pancreatic function, but it is certainly not the only one. Olmsted [22] has recently obtained observations, by using methods identical with ours, that point out clearly the importance of undernutrition in reducing pancreatic activity.

The significance of alterations in the bile pigments of the duodenal contents has been fully discussed in earlier communications.[23] Briefly it may be said that increased amounts of bilirubin, urobilin or urobilinogen in the bile excreted into the duodenum are abnormal, and indicate one of two things, increased blood destruction or abnormal liver function. In cases of diabetes the first factor obviously may be disregarded. Any marked increase in the elimination of one or several of the before mentioned pigments may probably be taken, therefore, as evidence of disturbed liver function. It has already been indicated that we do not know the exact nature of the disturbance of liver function that may exist in this disease. It may logically be assumed, however, that any alterations noted in bile pigment elimination in diabetes mellitus may properly be interpreted as an indication of an alteration in total metabolic activity of the hepatic parenchyma. This

22. Olmsted, W. H.: Department of Internal Medicine, Washington University, St. Louis; personal communication.

23. Jones, C. M.: Blood Pigment Metabolism and Its Relation to Liver Function, Arch. Int. Med. **29**:643 (May) 1922; Jones, C. M., and Minot, G. R.: Infectious (Catarrhal) Jaundice. An Attempt to Establish a Clinical Entity, Boston M. & S. J. **189**:531 (Oct. 18) 1923.

metabolic change is most likely a change associated with glycogen production, storage and mobilization, and any change in pigment metabolism is simply a reflection of alterations in the general liver processes. Examination of Table 1 shows that 67 per cent. of the patients showed increased elimination of bilirubin; 56 per cent. showed abnormal amounts of urobilin, and 40 per cent. were putting out excessive amounts of urobilinogen. The order of frequency in which the abnormalities were noted in the three pigments is entirely in keeping with previous results. We have found, in studying other types of cases, that those with alteration in hepatic function most frequently present abnormalities in bilirubin elimination, next in that of urobilin, and lastly in urobilinogen. Seventy-six per cent. of the cases in this series showed abnormally high values for one or more of the pigments. Such a high percentage of abnormal values for bile pigment elimination would seem to be clear evidence of an abnormal liver function or activity in diabetes mellitus. Whatever the exact nature of this disturbance of hepatic function may be, it appears likely that there is an abnormal, undesirable and probably inefficient type of work done by the liver in this disease.

That there is an intimate relation between the abnormalities in pancreatic and liver function already noted would seem indicated by the fact that in one-third of the cases there were marked changes in both enzyme and bile pigment estimations.

An attempt has been made in Tables 2, 3 and 4 to analyze some of the alterations noted in enzyme or pigment estimations. Obviously it is impossible to determine precisely the effect of any given variable, but the general trend of the influence of various factors may perhaps be indicated. In Table 2 the usual diabetic variables have been considered. Variations in sex, age, duration of the disease, and the fasting blood sugar on the day of examination seemed to exhibit no characteristic changes from the general percentages, either in pancreatic enzyme activity or bile pigment elimination. In considering the effect of diet, however, it is evident that those cases having a very low caloric intake per kilogram of body weight showed the most frequent occurrence of abnormal bile pigment elimination. Conversely, those patients on the highest caloric intake showed the fewest abnormalities. It is also of interest to note that all of those patients who were on an intake of less than 10 calories per kilogram of body weight showed abnormal pigment values, and these pigment abnormalities were among the most marked of any in the series. It seems logical to assume, therefore, that starvation or existence on a very low calory diet is associated with a marked increase in liver activity. This increase may be due to the mobilization of available fat and glycogen. Such a process invariably

takes place during inanition, and is associated with an actual loss of liver substance.

From an analysis of our figures it is difficult to determine the influence of insulin therapy on liver and enzyme activity. It would appear, however, that, when a sufficient quantity of insulin is given over a long enough period of time, there may be a reduction in the number of cases showing abnormalities in enzyme activity, bile and pigment metabolism. This may be due to an actual increase in food intake, or to a general improvement in tissue function following the use of insulin.

Table 3 represents an attempt to analyze the effect of certain complicating factors on hepatic and enzyme activity. Recent or existing acidosis, sepsis, gangrene, and the presence of marked arteriosclerosis seemed to have little or no special effect on the general percentage of abnormalities in the external pancreatic secretion. The presence of cholelithiasis, also, apparently exerted no characteristic influence on abnormal findings of enzyme activity, but all of the cases of probable pancreatitis showed marked reduction in digestive action of the duodenal contents. The cases of hemochromatosis all showed a similar change. Such findings are not surprising; on the contrary, it was to be expected that diseases directly associated with pathologic changes in the pancreas or biliary tract should exhibit such abnormalities.

An explanation of the bile pigment findings shown in Table 3 is somewhat difficult. Apparently, during an existing acidosis there is less change in liver activity than in the period immediately following. Sepsis has usually been associated with increased bile pigment elimination, and it was to be expected that cases with infection or general gangrene should show some increase in pigment estimations. The percentage of cases of cholelithiasis showing pigment abnormalities was the same as that of the entire group. The two rather striking exceptions to the average are the cases of pancreatitis and those of hemochromatosis. The results in the first group are not particularly surprising on account of the intimate relation between the pancreas and biliary tract. The absence of abnormal bile pigment values in the three cases of hemochromatosis was a distinct surprise, and, with our present lack of knowledge, suggests no ready explanation. An interesting conjecture is indicated, however, by the known pathology of this rare disease. Heavy pigment deposits always occur in the liver in hemochromatosis, and such deposits are largely composed of hemosiderin. Hemofuscin is also deposited in large amounts in the connective tissues. It is entirely possible that the lack of an increase in bile pigment elimination in this disease is due to the fact that any excess pigment, metabolized in the liver or elsewhere in the body, is retained in these pigment deposits.

A detailed theoretical discussion has been made of the various data outlined in Tables 2 and 3. We realize that the number of variable factors in any given case of diabetes render hypotheses based on findings in such small groups of cases extremely difficult of proof. Our intention has been only to indicate certain tendencies which appear to exist in relation to different factors in the disease. We realize that any accurate interpretation of the figures in these two tables is, of necessity, impossible.

Table 4 is of interest with regard to the probable effect of acidosis on the general body functions. Examination of the table indicates clearly that in these five cases there was a very striking improvement, both in enzymatic activity and liver function, following recovery from acidosis. With the single exception of the amylolytic activity, all the determinations showed a marked change in the direction of normalcy. Failure of this single enzyme to vary with the other two has been apparent throughout the study, and we are unable to assign any explanation to the behavior of this particular function of the pancreas. It will be noted that urobilinogen, which was the last to show abnormal changes in the presence of liver disturbance (Table 1), was the first pigment to return to normal, during the period of recovery from acidosis. Such a finding would seem to indicate that the presence of increased amounts of urobilinogen in the bile offers the surest evidence of liver disturbance that may be obtained by a study of the bile pigments, although increases in urobilin and bilirubin are undoubtedly of similar significance.

From the changes outlined in Table 4, one is forced to conclude that in severe acidosis there is a serious functional change in the liver and pancreas, as well as in other tissues of the body. In the case of the external secretion of the pancreas and the bile, the shift from very abnormal figures to normal during the recovery from acidosis is almost as striking as the well known changes that occur in the blood. That even mild acidosis is a serious condition for tissue function is almost universally accepted, but the truth of the statement is rendered all the more evident from findings similar to the above.

CONCLUSION

In closing, it is important that we consider briefly the relation of gallbladder disease to diabetes. In a previous paper,[19] satisfactory evidence has been presented of the value of duodenal analysis in the diagnosis of gallstones. Abnormal amounts of cholesterin or bile pigment crystals in the duodenal sediment were shown to be present in the vast majority of cases of cholelithiasis, and only rarely in other diseases. As an aid in the diagnosis of gallstones, we have found such

evidence extremely satisfactory. Examination of Table 5 shows that eleven patients, or 19 per cent. of the series, showed the characteristic findings of cholelithiasis, as judged by the foregoing criteria. In addition, two other patients (Cases 30 and 53) gave a history of previous operation for cholelithiasis, with the finding of stones at exploration. A third patient (Case 55) gave an absolutely characteristic story of recurring gallstone attacks, with known jaundice on two occasions, and a fourth patient (Case 22) had been previously diagnosed as cholelithiasis in this hospital, but operation was deferred. There is thus a total of fifteen cases, or 22 per cent. of the series, in which gallbladder disease may fairly be assumed to have existed. Such a percentage is much higher than that usually given as the incidence of cholelithiasis in diabetes. It is true that a group of sixty-eight diabetic patients is too small for statistical purposes. We are inclined to believe, however, that the generally accepted figure of from 6 to 7 per cent., as the incidence of gallstones in diabetes, is far too low. A more logical interpretation of the foregoing figures is possible if we also take into account the age of those patients giving evidence of cholelithiasis. The average age of these patients was 51 years, only one patient being under 45. A justifiable conclusion from such data would seem to be that, in the group of adult diabetic patients, the occurrence of gallstones is frequent. An attempt to determine the exact incidence in diabetic patients over 40 years of age from the series of cases here presented would be injudicious. It may not be unreasonable to suggest, however, that in patients with diabetes mellitus over the age of 30, cholelithiasis may well be an associated feature in at least a fifth of the cases. From our knowledge of the pathology of cholelithiasis it is interesting to consider if this relation to diabetes is an etiologic one. Joslin's figures, which take into account the probable onset of symptoms of cholelithiasis and the subsequent onset of diabetic symptoms, would seem to indicate that gallbladder disease is the cause of the pancreatic disturbance. Findings like those recently reported by Eustis,[24] as well as the evidence presented at the beginning of this paper, are also strongly in favor of such a view. In Eustis' thirty-six cases of so-called alimentary glycosuria, fifteen showed cholelithiasis. Six of these cases later presented symptoms of definite diabetes. If the etiologic rôle of gallbladder disease is accepted, we are inclined to agree with Joslin that not only in diabetic, but also in nondiabetic patients, the existence of cholelithiasis is an indication for surgical interference. In the case of nondiabetic patients, surgical intervention may reasonably be advised as a preventive measure against the subsequent development of diabetes.

24. Eustis, A.: Relation of Gallbladder Disease to Diabetes, New Orleans M. & S. J. **75**:449 (Feb.) 1923.

SUMMARY

1. An unselected group of sixty-eight diabetic patients has been examined for evidence of alteration in pancreatic or hepatic activity. Pancreatic enzyme activity was diminished in nearly one-half of the cases. Bile pigment elimination in the duodenal contents was abnormally high in about three-fourths of the cases. In nearly one-third of the cases there were associated enzyme and pigment abnormalities. The greatest alterations in enzyme activity were noted in the lipolytic and proteolytic ferments.

2. These findings are taken to indicate that in diabetes mellitus there is a marked alteration in the external secretory activity of the pancreas and in the hepatic function. The enzyme abnormalities are probably due to associated anatomic and functional changes in the acinar tissue of the pancreas. Undernutrition may play a part in the production of such changes, but it is not the sole cause. Such alterations in pancreatic or liver function may well contribute to the symptomatology of diabetes. It is suggested that the diminution of enzyme activity may result in disturbances due to improper digestion of fat and protein.

3. Undernutrition, of the several variable factors entering into an uncomplicated case of diabetes, evidently places the greatest strain on the liver, and results in striking bile pigment abnormalities. Undernutrition should therefore be avoided, on account of its undesirable effects on liver and pancreatic function.

4. Efficient insulin therapy, with its associated increase in food intake and improvement of tissue function, seems to be associated with a reduction in pancreatic and hepatic abnormalities.

5. Cases of diabetes complicated by pancreatic disease were found to be associated with an increased frequency of pancreatic enzyme disturbance. Sepsis, gangrene, and pancreatitis seemed to be predominating factors in producing an alteration in bile pigment metabolism. All three cases of hemochromatosis showed a reduction in enzyme activity, but no abnormality in pigment elimination.

6. Acidosis produces a marked disturbance of pancreatic enzyme activity and liver function. The improvement in pancreatic and hepatic function, as measured by changes in the enzyme activity and bile pigment elimination, following recovery from acidosis, is very striking, and illustrates the degree to which acidosis affects all bodily functions.

7. Cholelithiasis, as diagnosed by examination of the duodenal sediment, occurred in 19 per cent. of the cases in this series. The sediment findings by which such a diagnosis can be made are characteristic, and have been previously described. In addition, several other

patients had histories or operative findings consistent with the diagnosis of gallstones. The average age of this group of cases, constituting 22 per cent. of the entire series, was about 51. We believe that the existing figures for the incidence of gallstones in diabetes is far too low. It would not be surprising to find that at least one-fifth of all diabetic patients over 40 have an associated cholelithiasis.

8. In adults, cholelithiasis is probably one of the most important etiologic factors in diabetes mellitus. For this reason, it would seem that surgical intervention in all cases of gallbladder disease, in the absence of other complication, is indicated as a means of preventing the occurrence of diabetes, or of relieving an existing diabetes.

THE RESPIRATORY ORGANS IN HEALTH AND IN DISEASE

XVI. A COMPARISON OF VITAL CAPACITY STANDARDS IN THREE THOUSAND FIVE HUNDRED AND THIRTY-FOUR MALE UNIVERSITY STUDENTS *

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In spite of the large amount of recent work done on the many aspects of the subject of the vital lung capacity, disagreement still exists as to the best choice of standards for predicting the normal capacity of a given individual. The recent interest of thoracic surgeons in the use of vital capacity measurements as an aid to determine surgical risk adds another group to the large number of clinicians already seeking suitable normal standards for use in this work. Hutchinson's [1] pioneer work led to the belief that the standing height was the best measurement on which to base calculations of lung capacity. Peabody and Wentworth [2] preferred height measurements in their estimations. Lundsgaard and Van Slyke [3] advocated the use of certain chest dimensions. West [4] found surface area most reliable. Dreyer [5] prepared formulas for estimating vital capacity from height, weight, stem height and chest circumference, and these have been widely used. Dublin [6] questions the accuracy of all Dreyer's formulas, but finds the one based on weight most nearly correct. It has been established that the lung capacity

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* Read before the medical staffs of the Lymanhurst School and Hospital for Tuberculosis Children and the Parkview Sanatorium, June 24, 1924.

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varies with sex, age, weight, height, surface area, chest circumference, sitting height, previous occupation, state of health and past history of certain diseases. With so great a number of variables, the difficulty of obtaining enough constants to establish normal variations in lung capacity is apparent. The best opportunity of solving this difficulty appears to lie in observing large numbers of persons in whom the variables can be carefully estimated. The examination of college students seems to offer a good field for this purpose.

In most previous studies of this kind, it has been assumed that all the individuals of the series were normal from the fact that they were not disabled. In a very few instances, vital capacity measurements on large numbers of supposed normal persons have been reported following a cursory physical examination to establish this normality. Hutchinson [1] describes no special method of selecting known normals in his series of 3,000. Schuster [7] reported vital capacity measurements on 959 Oxford students, but did not detail a method of selecting the normals. Hewlett and Jackson's [8] series of 400 students "were active and showed no evident signs of disease." In West's [4] series, no exceptional measures to exclude abnormals are reported. In our previous series [9] of 1,304, no attempt was made to select absolute normals, but the range of the vital capacity distributions agreed fairly well with those reported as normals by others. In this series, demonstrable cause for low lung capacity was found in 106 of the 186 students below 90 per cent. of normal.

Experience has convinced us that a number of physical factors not uncommon among college students, as well as a history of certain past diseases, will lower the lung capacity. It has been shown,[10] for instance, that a group of persons giving a past history of pleurisy, even without regard to the type of pleurisy, will show a much lower vital capacity as a whole than a group without such history. The frequency with which extensive fibrinous adhesions of the pleural membranes are encountered at necropsy, and the relative infrequency of a past history of pleurisy even in carefully taken histories, illustrates the difficulty of selecting normal individuals in this single instance. Lemon and Moersch [11] enumerate many factors not ordinarily associated with pulmonary dis-

7. Schuster, E.: First Results from the Oxford Anthropometric Laboratory, Biometrika **8**:40, 1911.

8. Hewlett, A. W., and Jackson, N. R.: The Vital Capacity in a Group of College Students, Arch. Int. Med. **29**:515 (April) 1922.

9. Shepard, W. P., and Myers, J. A.: A Preliminary Study of the Vital Capacity in College Students, Journal-Lancet, **43**:355 (July 15) 1923.

10. Shepard, W. P.: The Effect of Certain Past Diseases on Vital Capacity, Arch. Int. Med. **33**:185 (Feb.) 1924.

11. Lemon, W. S., and Moersch, H. J.: Factors Influencing Vital Capacity. Arch. Int. Med. **33**:136 (Jan.) 1924.

ease which tend to lower the vital capacity. Acute respiratory infection, hernia, spinal curvature, unrecognized pulmonary and cardiac disease, overweight, even if slight, and other conditions must be ruled out in selecting a series on which to base standards for vital capacity measurements. Students from China, Japan, India and the Philippines frequently show low vital capacities, in our experience. A lowered vital capacity in Chinese students has been reported by Foster and Hsieh,[12] who suggest a racial factor as another possible variable.

This study was therefore undertaken with a view to comparing the various standards of estimating the vital capacity in a large group of normal young men.

METHODS

During the fall entrance examinations of the last three years at the University of Minnesota, the students' health service has paid particular attention to vital capacity measurements. The complete records of 3,534 male sudents were available for study. Vital capacity was measured by means of a Sanborn water spirometer. Time was taken to explain the instrument to each student, and to allow from three to five trials before recording the maximum reading. Many students having a low reading without apparent cause were rechecked at a later date, in an effort to secure readings of certain accuracy. All measurements were taken with the student stripped. Chest circumference was measured at the nipple line with the student's back to a mirror so that the tape might be kept horizontal. A measurement was recorded at full inspiration and again at full expiration and the computed mean used for further calculations, after the method of Gray. Sitting height was measured carefully, as described by Dreyer, with the student sitting on a table, the sacrum held firmly against a rule on the wall and tne knees drawn up. The condition of the student's health was determined with a fair degree of accuracy by fourteen medical examiners of our own staff, who were given between two and two and one-half hours for each student examined. The occurrence of all important past diseases was noted in the history sheet filled out by the student under competent supervision. By means of Myers'[13] tables, based on Dreyer's and West's formulas, vital capacity was computed in percentage of normal according to the individual weight, height, chest circumference, sitting height and surface

12. Foster, J. H., and Hsieh, P. L.: The Vital Capacity of the Chinese; an Occupational Study, Arch. Int. Med. **32**:335 (Sept.) 1923.

13. Myers, J. A.: Studies on the Respiratory Organs in Health and Disease, VIII, A Method for Quickly Obtaining the Percentage of an Individual's Theoretical Normal Vital Capacity of the Lungs, Am. Rev. Tuberc. **7**:161 (May) 1923; How Much Should Your Lungs Hold? J. Outdoor Life, November, 1923; The Vital Capacity of the Lungs (to be published).

area. Finally, the average of these five determinations was noted. The clerical error in the method employed was less than 2 per cent.

The records were then analyzed with a view to selecting those men in whom no physical defect having a possible bearing on vital capacity could be detected, and those having no past history of pulmonary or cardiac disease. This left only 1,641 who were considered normal. Among those excluded as abnormal and grouped for special study were 376 students giving a history of pneumonia; 333 who were 10 per cent. or more overweight for their age and height, according to life insurance standards, 111 giving a history of pleurisy or in whom physical findings of pleurisy

TABLE 1.—*Distribution of Vital Capacity Readings by Various Standards in One Thousand Six Hundred and Forty-One Normal Men University Students*

Percentage of Normal Vital Capacity	Weight		Height		Stem Height		Surface Area		Chest Circumference		Average	
	Cases	Per Cent.	Cases	Per Cent.	Cases	Per Cent.	Cases	Per Cent.	Cases	Per Cent.	Cases	Per Cent.
Under 70	...	.....	...	.....	...	.....	...	.....	...	.....	...	.....
70 to 74	...	.....	8	0.49	1	0.06	4	0.24	11	0.67	...	.....
75 to 79	5	0.31	15	0.92	3	0.18	12	0.73	40	2.44	8	0.49
80 to 84	12	0.73	44	2.68	15	0.92	29	1.77	122	7.43	16	1.10
85 to 89	38	2.32	122	7.43	50	3.05	122	7.43	213	12.96	74	4.51
90 to 94	192	11.70	231	14.08	98	5.97	225	13.70	317	19.30	200	12.20
95 to 99	242	14.75	272	16.57	160	9.75	295	17.98	289	17.60	312	19.00
100 to 104	317	19.33	294	17.91	231	14.05	296	18.03	256	15.60	326	19.88
105 to 109	239	14.56	205	12.49	232	14.12	233	14.20	148	9.02	246	15.00
110 to 114	233	14.20	178	10.85	260	15.83	181	11.02	113	6.88	223	13.60
115 to 119	171	10.42	131	7.98	203	12.35	121	7.37	75	4.57	143	8.72
120 to 124	107	6.52	88	5.36	206	12.55	83	5.06	38	2.32	61	3.72
125 to 129	46	2.80	37	2.25	168	10.22	27	1.64	17	1.03	25	1.52
130 and over	39	2.38	16	1.10	14	0.85	13	0.79	2	0.12	7	0.43
Totals..	1,641	100.02	1,641	100.11	1,641	99.90	1,641	99.96	1,641	99.94	1,641	100.17

were detected. There were thirty-six students excluded as having history or physical findings, on subsequent examination, of pulmonary tuberculosis. About one third of these had not previously known of the existence of the disease. Thirty-four students showed definite signs of organic heart disease, but were apparently well compensated at the time. These were grouped for special study. A special group was formed of 574 students who were 10 per cent. or more underweight, without any evidence of organic defect or past history of pulmonary or cardiac disease. Other conditions encountered and for which the students were excluded as abnormal were: acute respiratory infection; hernia, which was frequently found to lower the vital capacity; cicatrices from wounds about the thorax and shoulder girdle; goiter; paralyses, with atrophy of the muscles of the spine or thorax; congenital absence of the pectorals; amputations; developmental abnormalities of the bony thorax; marked spinal curvature, and chronic diseases, such as nephritis.

The ages varied from 15 to 35 years. The average and the modal age was 18. Hutchinson,[1] Wintrich,[14] Cornet [15] and Pratt [16] agree that age does not lower vital capacity until from 30 to 35 years are reached.

RESULTS

The incidence distribution of vital capacity readings in the 1,641 students who were considered normal is shown in Table 1 and illustrated in Chart 1. In studying the summation curves in this figure, it will be seen that any agent tending to lower the vital capacity readings shifts the entire curve to the left. From this figure, it will be seen that the computations of vital capacity by means of surface area and standing

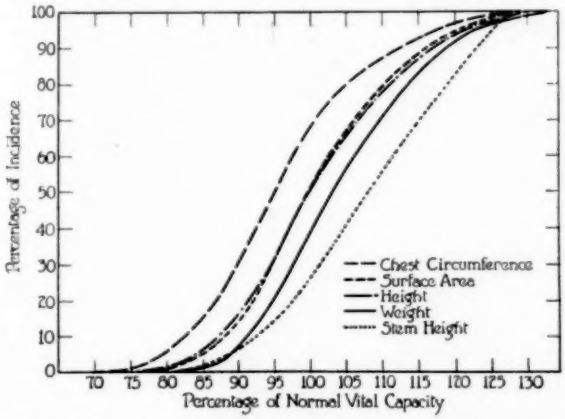

Chart 1.—Vital capacity readings by various standards of 1,641 normal men university students.

height almost coincide. The weight formula of Dreyer is practically parallel to the surface area and the height curves and but slightly to the right. Estimations of vital capacity, according to stem height, by the use of the formula employed here give readings too high in comparison to the formulas based on surface area, height and weight. Estimations according to chest circumference, on the other hand, give readings too low. It also will be seen that this group of normal young men showed more vital capacities above 100 per cent. normal than below. In Table 1,

14. Wintrich, M. A.: Einleitung zur Darstellung der Krankheiten der Respirationsorgane, Handb. d. spec. Path. u. Therap. Virchow, 1854.

15. Cornet, J.: Einige spirometrische Beobachtungen nebst einem Rückblick auf. die bis jetzt aufgestellten methoden zur Bestimmung der physikalischen Vitalcapacitat, Inaugural Dissertation, München, 1884.

16. Pratt, J. H.: Long Continued Observations on the Vital Capacity in Health and Heart Disease, Am. J. M. Sc. **164**:819 (Dec.) 1922.

nearly 70 per cent. of the total number are more than 100 per cent., or normal vital capacity, according to the weight formula, and 35 per cent. are more than 110 per cent. normal. Only about 3 per cent. are below 90 per cent. normal vital capacity. It would therefore seem that, when applied to selected normal young men, the vital capacity standards commonly in use are slightly too low. Surface area, standing height and weight standards most nearly coincide. The chest circumference standard is too low and the stem height standard too high compared to the others.

This is in agreement with the findings of West,[4] who advocated surface area as the best standard on which to estimate lung capacity. In his series of 129, only 5.5 per cent. were found to have capacities less than 90 per cent. normal. He also stated that Dreyer's weight formula practically agreed with the surface area standard. Hewlett and Jackson [8] reported better correlation between surface area and vital capacity than between height or weight and vital capacity. In their series of 400, however, 20 per cent. of the students fell below 90 per cent. normal lung capacity. In our previous series,[9] using the weight formula of Dreyer and taking what appeared from superficial observation to be normals, 14 per cent. fell below 90 per cent. normal. In studying the vital capacity in old age with various standards, Myers and Cady [17] found a close agreement between the estimations based on surface area, standing height and weight; while those based on sitting height gave readings 10 per cent. higher in men. In studying the vital capacity measurements of 738 college women, Boynton [18] found that the weight and surface area standards nearly coincided, while the stem height estimation gave readings too low and was subject to considerable error. Hewlett and Jackson [8] suggest that present standards are too low when applied to a selected group of students.

STUDY OF ABNORMALS

In order to emphasize the necessity of careful selection of normals when comparing standards for the estimation of vital capacity, certain of the larger groups of those rejected as abnormal were subjected to special study. The distribution of the vital capacities of these groups is given in Table 2. The average of all five vital capacity determinations (weight, height, surface area, stem height and chest circumference) was used in these groups in order to make the results comparable to those previously reported.[10]

17. Myers, J. A., and Cady, L. H.: Studies on the Respiratory Organs in Health and Disease, XIII, The Effects of Senility on the Vital Capacity of the Lungs, Am. Rev. Tuberc. **9**:57 (March) 1924.

18. Boynton, Ruth E.: A Comparison of Normal Standards for the Vital Capacity of the Lungs of Women, Arch. Int. Med. **33**:292 (March) 1924.

Three hundred and seventy-six students gave a history of having had pneumonia at some time. Neither the type of pneumonia nor its gravity were determined, but, on the whole, the student's memory of the disease would be better in the more serious cases. The vital capacities of this group are shown in the first column of Table 2, and illustrated by means of summation curves in comparison to the normal in Chart 2. The group, as a whole, shows a slight but definite lowering of the vital capacity throughout.

One hundred and eleven students gave a history of pleurisy. Again, the type and gravity of the disease was undetermined, but, judging from the vital capacities of this group as seen in the second column of Table 2

TABLE 2.—*Distribution of Vital Capacity Readings in Certain Groups Considered Abnormal*

Percentage of Normal Vital Capacity	Pneumonia		Pleurisy		Tuberculosis		Cardiac Disease		Overweight		Underweight	
	Cases	Per Cent.	Cases	Per Cent.	Cases	Per Cent.	Cases	Per Cent.	Cases	Per Cent.	Cases	Per Cent.
Under 70	...	.....	...	....	2	5.56	...	.....	...	.....	...	.....
70 to 74	2	0.53	2	1.80	1	2.78	1	2.94	3	0.90	2	0.35
75 to 79	6	1.60	4	3.60	1	2.78	...	.....	6	1.80	3	0.52
80 to 84	10	2.66	8	7.20	8	22.20	3	8.83	11	3.30	15	2.62
85 to 89	35	9.31	10	9.00	1	2.78	8	23.50	19	5.70	28	4.88
90 to 94	43	11.43	16	14.40	8	22.20	2	5.88	48	14.40	86	15.00
95 to 99	68	18.10	21	18.90	4	11.10	1	2.94	52	15.61	82	14.30
100 to 104	68	18.10	16	14.40	4	11.10	6	17.65	56	16.80	125	21.80
105 to 109	66	17.55	17	15.30	3	8.33	5	14.70	55	16.50	86	15.00
110 to 114	40	10.64	9	8.10	1	2.78	6	17.65	36	10.80	60	10.45
115 to 119	25	6.65	4	3.60	2	5.56	1	2.94	30	9.00	61	10.63
120 to 124	8	2.13	4	3.60	1	2.78	1	2.94	12	3.60	17	2.96
125 to 129	4	1.06	...	.....	...	.....	...	.....	3	0.90	8	1.40
130 to 134	1	0.27	...	.....	...	.....	...	.....	2	0.60	1	0.18
Totals..	376	100.03	111	99.90	36	99.95	34	99.97	333	99.91	574	100.09

and illustrated in Chart 2, the disease was serious enough in the group, as a whole, to cause a definite lowering of the vital capacity. The displacement of the curve to the left is nearly as great as in the small group with tuberculosis described below.

Thirty-six students gave histories or showed physical signs, on subsequent examination, of pulmonary tuberculosis. This included only those with definite physical findings or those who gave an unquestionable history. The effect of this disease on the vital capacity of the group as a whole is shown in the third column of Table 2, and illustrated in comparison with the normal in Chart 3. The lowering of the vital capacity is most pronounced in this group.

Thirty-four students gave unquestionable history, or showed definite physical signs of organic heart disease. In view of the effect of heart disease on vital capacity reported by Ulrich and Nathanson [19] and others,

19. Ulrich, H. L., and Nathanson, M. H.: The Vital Capacity of the Lungs in Cardiac Disease, Minnesota Med. **4**:721 (Dec.) 1921.

these were grouped for special study. It is noteworthy that all these individuals appeared to be in good health and about one-half the group had never suspected the existence of cardiac disease. In spite of the

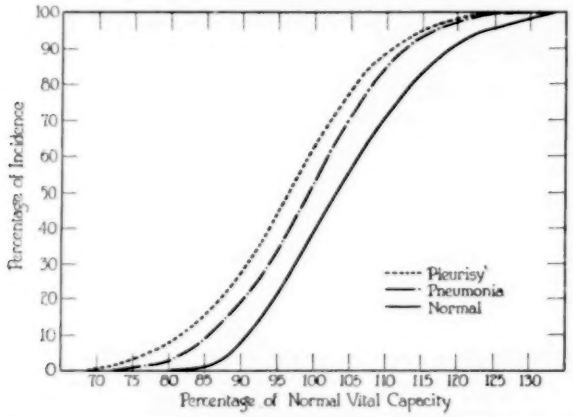

Chart 2.—Comparison between vital capacities of normal students and those giving histories of pneumonia and pleurisy.

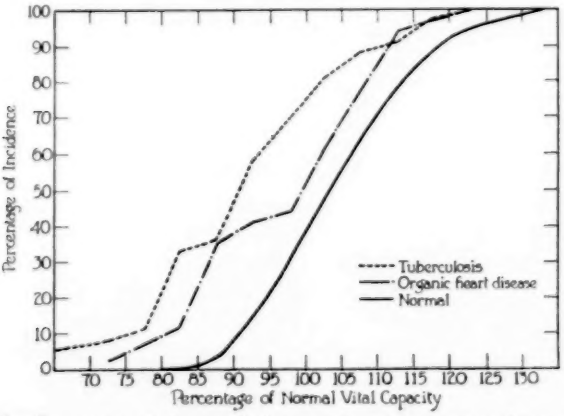

Chart 3.—Comparison between vital capacities of normal students and those with histories or physical signs of pulmonary tuberculosis or organic heart disease.

absence of symptoms or signs of decompensation or pulmonary congestion, this group, as a whole, showed a marked lowering of the vital capacity (Table 2, Chart 3).

The effect of obesity on the vital capacity has long been recognized. Since the degree of obesity required to lower the vital capacity is ques-

tionable, and it was desired to come well within the margin of safety in selecting the group of normals, all persons who were 10 per cent. or more overweight for their age and height, according to life insurance standards, were placed in a special group numbering 333 cases. Table 2 and Chart 4 show that the vital capacity of this group, as a whole, is slightly lowered. Obviously, this method excluded some of the big men who were not truly obese and who had unusually large lung capacity. The occurrence of marked obesity was rare in this age group.

There was a large group of 574 students who were 10 per cent. or more underweight, according to insurance standards, but who showed no definite organic defects. Obviously, being 10 per cent. underweight is not a serious defect at this age. In some members of the group, how-

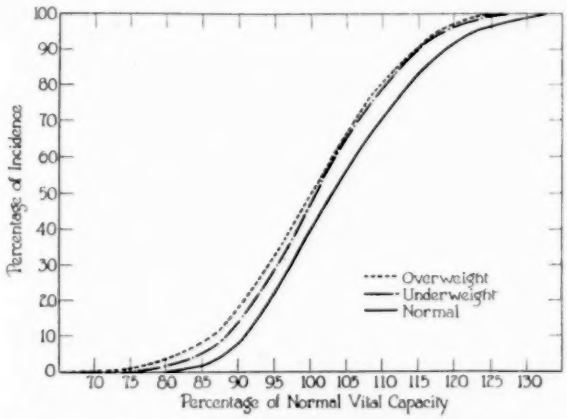

Chart 4.—Comparison between vital capacities of normal students and those 10 per cent. or more underweight or overweight.

ever, tuberculosis was suspected; others showed slight evidences of malnutrition; many were of the immature, nonrobust type. Hence, many could not be called normal, and the entire group was excluded. The vital capacities of this group were slightly lowered as shown in Table 2 and Chart 4.

When a curve is plotted to show the vital capacity of all those considered abnormal as compared to a curve for all those considered normal, the curve for abnormals is slightly but definitely lower throughout. The difference is small enough to bring up the possibility of its being negligible. It is believed, however, that when the size of the series is considered, together with the fact that the division between abnormals and normals can never be absolute, the importance of using great care in selecting normals as a basis for study is still apparent.

CONCLUSIONS

1. When applied to 1,641 carefully selected men university students, the commonly used standards for estimating vital capacity from surface area, height and weight practically coincide.

2. The estimation based on chest circumference gives readings considerably too low, while that based on sitting height gives readings too high.

3. When applied to this selected group, all standards give readings too high for a normal distribution.

4. Great care should be used in selecting normals for purposes of working out standards for the estimation of normal vital capacity.

THE PERMEABILITY OF HUMAN BLOOD CELLS TO CARBON DIOXID AND AMMONIUM HYDROXID IN SOLUTIONS OF SAME p_H *

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INTRODUCTION

The pathology of Bichat, more than a century ago, centered about organs and tissues and that of Virchow about cells, while that of today also concerns the contents of later morphologic units. Little is known of the great variety of complex processes and of obscure changes which occur within cells, but it is recognized that the regulation of their hydrogen ion concentration plays an important rôle in maintaining their normal activity. In contrast to the simplicity of determining the hydrogen ion concentration of the body fluids, it is perhaps impossible to determine it accurately for a part or the whole of living cells. It is a frequent assumption that cells are of the same hydrogen ion concentration as their surrounding medium. That this is not necessarily the case has been shown by Bethe,[1] Warburg,[2] Harvey,[3] and Jacobs [4] from studies made on sea urchin eggs and plant cells. Jacobs has clearly shown that the substances contained in the surrounding fluid, especially carbon dioxid and ammonium salts, have more influence on the p_H of structures within cells than does the actual p_H of their surrounding medium. The relative permeability of the cells of the human body to carbon dioxid is an important phase in the regulation of respiration, and

* From the medical service of the Collis P. Huntington Memorial Hospital of Harvard University. This paper is No. 37 of a series of studies on metabolism from the Medical School of Harvard University and allied hospitals. The expenses of this investigation have been defrayed by a grant from the Proctor Fund of the Medical School of Harvard University for the study of chronic diseases.

1. Bethe, A.: Die Bedeutung der Elektrolyten fur die rhythmischen Bewegungen der Medusen, Arch. f. d. ges. Physiol. **127**:219, 1909.

2. Warburg, O.: Ueber die Oxydationen in lebenden Zellen nach Versuchen am Seeigelei, Ztschr. f. physiol. Chem. **66**:305, 1910.

3. Harvey, E. N.: Studies on the Permeability of Cells, J. Exper. Zool. **10**:507, 1911.

4. Jacobs, M. H.: The Production of Intracellular Acidity by Neutral and Alkaline Solutions Containing Carbon Dioxid, Am. J. Physiol. **53**:457 (Oct.) 1920; to What Extent Are the Physiological Effects of Carbon Dioxid Due to Hydrogen Ions? ibid. **51**:321 (March) 1920; The Influence of Ammonium Salts on Cell Reaction, J. General Physiol. **5**:181 (Nov.) 1922. General Cytology, edited by E. V. Cowdry, Section 3, Chicago, University of Chicago Press, 1924.

the question arises whether or not different human cells have the same permeability for carbon dioxid and ammonia. An attempt to answer this question and to determine if Jacobs' results with sea urchin eggs are applicable to living human cells has been the object of the investigations reported here.

METHODS

The observations have been confined to the formed elements of the human blood since they offer conveniently accessible test cells. These observations concern mainly the white blood corpuscles and, to a lesser extent, the platelets. No information could be deduced concerning intracellular changes in the red blood corpuscles except with regard to internal structures, which they contain as a result of their immaturity.

In the first set of observations, a drop of blood was used without any anticoagulant. In the subsequent studies, large numbers of cells were obtained by sedimentation from venous blood to which crystalline sodium citrate was added.

The relatively nontoxic, vitally staining dye, neutral red, was used since it acts as an indicator at the p_H of the blood. The strength of the dye was varied from 0.04 to 0.1 per cent. in absolute alcohol in order to stain adequately the varying numbers of cells used.

Chemically clean, dust free, glass slides were flooded with the alcoholic solution of dye; the excess was quickly drained off, and the film allowed to dry. A drop of fresh blood was then taken on a clean glass cover slip, placed on the prepared slide, and sealed with petrolatum. This preparation was then kept in a warm stage at 37.5 C. After five minutes, the intracellular structures within the blood corpuscles which take up neutral red became uniformly stained. The influence on the cells of three solutions of the same p_H, but of different composition, was then tested. These three solutions were the same as those used by Jacobs,[4] and were prepared as follows:

1. Half molar sodium chlorid was brought to p_H 7.4 with a trace of sodium bicarbonate.

2. Half molar ammonium chlorid was brought to p_H 7.4 with ammonium hydroxid.

3. Half molar sodium bicarbonate was brought to p_H 7.4 with carbon dioxid gas.

These solutions were drawn under the cover glass and mixed with the blood, according to the requirements of the different experiments. In the group of experiments with masses of cells, larger quantities of the solutions were mixed with the "leukocyte cream" in test tubes.

All determinations of the hydrogen ion concentrations of fluids were made by color comparison with standard buffer solutions (prepared

according to the method of Palitzsch [5]) to which brom-thymol blue was added as an indicator.

The different experiments were repeated numerous times in order to verify the correctness of the observations.

OBSERVATIONS

1. *The Changes Within the Cells.*—An excellent study of blood cells stained with vital dyes has been made by Sabin,[6] so that only brief reference will be given here to the phenomena observed.

When fresh blood is stained with neutral red, the polymorphonuclear neutrophil leukocytes, within the course of a few minutes, show red staining particles that are definite, constant and characteristic structures within the cytoplasm. It is not possible to tell definitely whether these vitally staining granules are the same as those that become colored in a fixed preparation with Wright's stain. These vitally stained particles are constantly streaming through the cytoplasm as the cell puts out its blunt pseudopodia. If the cell is injured, it ceases to move, and the vitally staining granules take on brownian movement. If the cells die, their nuclei, which have previously been unstained, take up the dye, while the vitally staining granules in the cytoplasm lose much of their red color. The behavior of all other white blood cells is similar to that of the polymorphonuclear neutrophil leukocytes, and the number of red staining particles apparently varies with the number of granules observed in similar cells stained with Wright's stain.

Characteristically, the platelets contain several intracellular structures that stain brilliantly with neutral red. When the platelets are dead, the dye may permeate diffusely through their protoplasm, giving it the appearance of ground glass.

In a preparation of fresh blood stained with neutral red, there may be seen only a very faint staining of the mature red blood corpuscles. Normal immature red blood corpuscles may contain nuclei, reticulum, and granules (Isaacs [7]), and of these structures in the living cell only the reticulum stains with neutral red. This staining of the reticular substance was observed many times, and even the smallest portion quickly takes up the dye.

The fresh vitally stained specimens of blood were each subjected to one of the three solutions previously described, and their effect was

5. Palitzsch, S., quoted by Clark, W. M.: The Determination of Hydrogen Ions, Baltimore, Williams & Wilkins Co., 1920, p. 85.

6. Sabin, F. R.: Studies of Living Human Blood Cells, Bull. Johns Hopkins Hosp. **34**:277 (Sept.) 1923.

7. Isaacs, R.: Properties of Young Erythrocytes in Relation to Agglutination, and Their Behavior in Hemorrhage and Transfusion, Arch. Int. Med. **33**:93 (Feb.) 1924.

noted on the vitally staining intracellular structures. In Table 1 are shown the changes caused by the solutions in the color of the stained particles of living and dead polymorphonuclear neutrophil leukocytes and platelets, in the stained nucleus and partially stained cytoplasm of these cells when dead, and in the cytoplasm and reticulum of nucleated and nonnucleated erythrocytes.

The solution of sodium chlorid brought to p_H 7.4 with a trace of sodium bicarbonate caused no change in the color of any stained intracellular structures. The table shows, however, that when a living leukocyte is subjected to the solution at p_H 7.4 which contains ammonia, the reaction of the vitally stained intracellular particles becomes alkaline, while if the surrounding solution, also at p_H 7.4, contains carbon dioxid,

TABLE 1.—*Effect of Three Different Solutions of Same p_H on Structures of Blood Cells That Stain Vitally with Neutral Red*

Type of Cell Structure	Solution 1 Half Molar Sodium Chlorid and Trace Sodium Bicarbonate *p_H 7.4*	Solution 2 Half Molar Ammonium Chlorid and Ammonium Hydroxid *p_H 7.4*	Solution 3 Half Molar Sodium Bicarbonate and Carbon Dioxid *p_H 7.4*
Cytoplasmic granules of living polymorphonuclear neutrophil leukocytes and blood platelets	No change	Change to yellow; alkaline reaction	Change to scarlet; acid reaction
Nucleus and cytoplasmic granules of dead polymorphonuclear neutrophil leukocytes and blood platelets	No change	Change to scarlet; acid reaction	Change to yellow; alkaline reaction
Cytoplasm of nucleated and nonnucleated erythrocytes	No detectable change	No detectable change	No detectable change
Reticulum of nucleated and nonnucleated erythrocytes	No change	Change to scarlet; acid reaction	Change to yellow; alkaline reaction

the reaction of these granules becomes on the acid side of neutrality. The stained structures in the dead leukocytes and the reticulum of the immature red blood corpuscles, on the other hand, have an opposite reaction to the latter two solutions to that of structures within the living white corpuscles.

Observations made on polymorphonuclear eosinophil and basophil leukocytes, large mononuclear leukocytes, lymphocytes, and all forms of immature myeloid and lymphoid cells from cases of myelogenous and lymphatic leukemia showed that the vitally staining structures which they contain reacted in the same way to the test solutions as did the stained granules of the living neutrophilic leukocytes. The mechanical difficulties of exposing all the cells to the test solutions at the same moment were such that it was impossible to make any accurate quantitative comparisons on the time required to change the color of the stained particles. The impression was gained that the lymphocytes not only stained more rapidly with the dye but also reacted more quickly to the

test solutions than did the other white blood corpuscles. It is worthy of note that the behavior of all forms of white blood corpuscles to the test solutions is strikingly similar irrespective of either their morphologic differentiation or their degree of maturity.

The stained particles in the blood platelets reacted in the same fashion as the vitally stained granules of the living leukocytes, which is to be expected in view of the fact that the platelets are detached cytoplasmic fragments.

Changes in the color of adult erythrocytes when subjected to neutral red are so slight that they do not serve to indicate alterations in the hydrogen ion concentration. The adult red blood corpuscles are such important units in the scheme of hemal transportation that many attempts were made to introduce an indicator into their protoplasm for the purpose of observing their reaction. Numerous vital dyes were tried but, unfortunately, none sufficed for the purpose.

That the reticulum of the nucleated and nonnucleated erythrocytes should react to the test solutions in the same way as the stained structures of the dead leukocytes may be taken as evidence in favor of the view set forth by Key,[8] that the reticulum is an inactive substance which is precipitated by the action of certain vital stains. However, this observation may have no bearing on the nature of the reticular substance for the phenomenon may be due to a different character of the cell membrane of the erythrocytes from that of the living white blood corpuscles. Cytologically, the erythrocytes often are not considered as true cells, hence the semipermeable properties of their membranes may not be comparable to those of other living cells.

2. *The Changes in the Surrounding Medium.*—It has been observed that when blood cells stained with neutral red are subjected to solutions of the same p_H, but of different composition, the color reaction of those intracellular structures which take up the dye may be changed. It is logical to assume that the reaction of the surrounding medium should become altered as a result of the loss of those substances which penetrate the cells. The following experiment was devised to determine if this change could be demonstrated.

One cubic centimeter of the "leukocyte cream" from blood obtained from patients with chronic leukemia was placed in a warm glass centrifuge tube. Two cubic centimeters of the solution to be tested were mixed with these living cells. This preparation was at once centrifugated for three minutes at 1,000 revolutions per minute and the p_H of the resulting supernatant fluid was determined immediately.

8. Key, J. A.: Studies on Erythrocytes, with Special Reference to Reticulum, Polychromatophilia and Mitochondria, Arch. Int. Med. **28**:511 (Nov.) 1921.

This experiment was repeatedly done with each of the three test solutions, using white cells removed from the blood both soon and some time after its withdrawal from the body.

The results obtained were always consistent with those given in Table 2.

The time which elapses between the withdrawal of the blood from the body and the separation of the white corpuscles influences the results. When the cells are separated from the blood by centrifugalization as soon as possible after its removal from the blood stream and a uniform number immediately mixed with each of the three test solutions, the p_H of Solutions 1 and 3 is unchanged, while that of Solution 2 is slightly decreased. On the other hand, when the whole blood stands in an incubator at 37.5 C. for approximately two hours, until the cells have settled out, the solutions then, after contact with the cells, show a decrease in the p_H of Solutions 1 and 2 while that of Solution 3 remains

TABLE 2.—*Changes in the p_H of the Test Solutions Surrounding the Living White Blood Corpuscles*

Interval Between the Withdrawal of the Blood from the Body and the Separation of the White Cells	Solution 1 Half Molar Sodium Chlorid and Trace Sodium Bicarbonate p_H 7.4	Solution 2 Half Molar Ammonium Chlorid and Ammonium Hydroxid p_H 7.4	Solution 3 Half Molar Sodium Bicarbonate and Carbon Dioxid p_H 7.4
About 8 minutes	p_H 7.4	p_H 7.1	p_H 7.4
About 2 hours	p_H 6.8	p_H 6.8	p_H 7.4

unchanged. It is probable that the products of cellular metabolism collect in the cells, on standing, to a sufficient extent to cause the variation in the findings.

It will be recalled that the vitally stained particles in the living leukocytes were observed to have an acid reaction when subjected to the solution containing carbon dioxid. Apparently, this solution has lost acid which has penetrated the cells, and yet, after contact with large numbers of cells, no change in its p_H was demonstrated. In contradistinction, the penetration of ammonia from Solution 2 into the white blood corpuscles results in a demonstrable increase in acidity of this solution, and this acidity is more pronounced if the cells are sedimented by standing in an incubator. Solution 1 contains no substances capable of rapid penetration into the cells, and there is no change in its p_H other than that caused by the condition of the cells. These results can be explained by recognizing the difference in buffer properties of the three test solutions. Solution 1 (sodium chlorid plus sodium bicarbonate) changes its p_H with great ease on the addition of acid; Solution 3 (sodium bicarbonate plus carbon dioxid) resists strongly change of p_H

by the addition of acid; while Solution 2 (ammonium chlorid plus ammonium hydroxid) acts intermediately between these two. It seemed reasonable that since these solutions differ in their buffer properties, any changes which occurred as a result of the migration of ions into the cells might be demonstrated by titrating the solutions before and after they had been mixed with leukocytes. Consequently, each of the solutions was titrated to p_H 6.6 with hundredth normal hydrochloric acid, using phenol red as an indicator, both before and after the solutions had been in contact, for about four minutes, with white blood cells obtained as soon as possible from patients with leukemia. The results shown in Table 3 were obtained in each of several successive observations with cells of both lymphoid and myeloid origin.

TABLE 3.—*Amount of Hundredth Normal Hydrochloric Acid Required to Titrate 1 c.c. of Test Solution to p_H 6.6, Using Phenol Red as an Indicator*

Conditions of Experiment	Solution 1 Half Molar Sodium Chlorid and Trace Sodium Bicarbonate p_H 7.4	Solution 2 Half Molar Ammonium Chlorid and Ammonium Hydroxid p_H 7.4	Solution 3 Half Molar Sodium Bicarbonate and Carbon Dioxid p_H 7.4
Before mixing with the "leukocyte cream"	1.2 c.c. N/100 hydrochloric acid	1.75 c.c. N/100 hydrochloric acid	4.1 c.c. N/100 hydrochloric acid
After mixing with the "leukocyte cream"	1.1 c.c. N/100 hydrochloric acid	0.8 c.c. N/100 hydrochloric acid	4.9 c.c. N/100 hydrochloric acid

The results shown in Table 3 not only illustrate the relative buffer properties of the test solutions, but also give proof of the fact that the surrounding medium changes its titratable acidity when there is a change of color reaction of stained intracellular structures. It will be observed that there is little or no change in the solution containing sodium chlorid and sodium bicarbonate after it has been mixed with white blood cells, nor does this solution cause any detectable change in the color of those intracellular particles which stain vitally with neutral red. The solution which contains ammonia causes the vitally stained particles of living cells to become yellow, for it loses alkali when in contact with cells, and, hence, less acid is required to bring it to a p_H of 6.6. Finally, the penetration of carbon dioxid into the living leukocytes causes their vitally stained structures to change from red to scarlet, and leaves the third solution more alkaline, and, as a result, more hundredth normal hydrochloric acid is required to bring it to a p_H of 6.6.

COMMENT

The experiments demonstrate for human blood cells what Jacobs [4] has shown holds true for sea urchin eggs and plant cells, namely, that intracellular acidity and alkalinity may be produced by solutions of the

same p_H but of different composition. No distinct differences could be detected between any of the known forms of mature and immature living white blood corpuscles or blood platelets in their reaction to the three solutions. However, the reaction for dead cells and for the reticulum of erythrocytes was the reverse of that for living white blood cells.

It is presumed that the presence of carbon dioxid and ammonia in these solutions is responsible for the changes that occur. In all biologic phenomena involving carbon dioxid and ammonia for their production, there is always sufficient water present to bring about the dissociation of these substances. Carbon dioxid in aqueous solution behaves as a weak acid for its dissociates as follows:

$$CO_2 + H_2O \rightleftarrows H_2CO_3$$
$$H_2CO_3 \rightleftarrows H^+ + HCO_3^-$$
$$HCO_3^- \rightleftarrows H^+ + CO_3^=$$

The partial hydrolysis of the acid salt ammonium chlorid in aqueous solution produces ammonium hydroxid, and, in the test solution used, a trace of the latter is added to secure a reaction for the whole of p_H 7.4. Sodium hydroxid, aside from the equilibrium which it maintains with ammonia, dissociates into ammonium and hydroxid ions. There is insufficient evidence at hand to determine definitely what forms of carbon dioxid and ammonia penetrate the cells and what forms cause the changes observed. Therefore, when the terms carbon dioxid and ammonia are used here, they should be interpreted in their broadest sense to include any of their dissociation products in aqueous solutions.

The experiments that have been performed demonstrate not only that the p_H of intracellular structures, as shown by their color changes, may be changed in either direction or unchanged by solutions of the same p_H, but they also show that the change of reaction of these solutions is in the opposite direction to those within the cells. The solutions become more acid when the stained intracellular particles become alkaline and vice versa. This phenomenon of the cellular reaction being opposite to that of the surrounding medium occurs in the physiologic processes of the production of gastric juice and the secretion of urine. Bernard,[9] by the use of the Prussian blue reaction, demonstrated that the gastric glands were not acid. Harvey and Bensley,[10] by means of the injection of vital dyes, and Chambers,[11] by microdissection studies,

9. Bernard, Claude: Leçons sur les propriétés physiologiques des liquides de l'organsime, Paris, 1859, p. 375.

10. Harvey, B. C. H., and Bensley, R. R.: Upon the Formation of Hydrochloric Acid in the Foveolae and on the Surface of the Gastric Mucous Membrane and the Nonacid Character of the Contents of Gland Cells and Lumina, Biol. Bull. **23**:225, 1912.

11. Chambers, R.: Microdissection Studies on the Physical Properties of Protoplasm, Lancet-Clinic **123**:363, 1915.

have shown that the parietal cells of the gastric glands are alkaline when there is an active production of an acid gastric juice. Stieglitz [12] has recently demonstrated that the cells of the kidney tubules, which are aiding in the secretion of an acid urine and are nourished by blood which is nearly neutral, are themselves alkaline in reaction. This difference in reaction between cells and fluids may be an important phase of the mechanism for the control of the hydrogen ion concentration of the organism. In any event, it may be conclusively stated that the presumption that the p_H of the surrounding medium is always the same as the p_H of the cells is erroneous and untenable.

The results that have been obtained indicate that carbon dioxid and ammonia penetrate human blood cells more easily than do the other substances present in the solutions. This is in accord with previous knowledge concerning these substances. It is probable that, since carbon dioxid and ammonia are formed in the body, the physiologic results which they produce, in part, may be attributed to their property of permeability. In no way is it wished to convey the idea that, by demonstrating a change of p_H of the vitally staining structures in blood cells, there has been demonstrated a change of p_H of the cytoplasm. The living cytoplasm, as it is ordinarily conceived, contains amphoteric electrolytes, which would resist change in p_H. Moreover, as Fischell,[13] M. R. Lewis,[14] W. H. Lewis [15] and Prigosen [16] have maintained, neutral red furnishes data only in regard to certain preformed bodies existing within the cells. That these stained particles are chemically fixed and are not a part of the living cytoplasm has been put forth by W. H. Lewis [15] and by Bechold.[17] The statement of M. R. Lewis,[14] that "it is impossible, so far as I know, to determine the hydrogen ion concentration of the living cell," clearly and correctly expresses the difficulties of the problem. This does not detract from the importance of the fact that one cannot predict the p_H of structures within the cell from the p_H

12. Stieglitz, E. J.: Histologic Hydrogen Ion Studies of the Kidney, Arch. Int. Med. **33**:483 (April) 1924.

13. Fischel, A.: Untersuchungen über vitale Farbung, Anat. Hefte **16**:415, 1901.

14. Lewis, M. R.: The Effect of Certain Vital Stains Upon the Development of the Eggs of *Cerebratulus lacteus, Echinorachnius parma* and *Laphius piscatorius*, Anat. Rec. **13**:21, 1917. Reversible Gelation in Living Cells, Bull. Johns Hopkins Hosp. **34**:373 (Nov.) 1923.

15. Lewis, W. H.: Degeneration Granules and Vacuoles in the Fibroblasts of Chick Embryos Cultivated in Vitro, Bull. Johns Hopkins Hosp. **30**:81 (April) 1919.

16. Prigosen, R. E.: The Formation of Vacuoles and Neutral Red Granules in Connective Tissue Cells and Blood Cells Observed Under Abnormal Conditions, Bull. Johns Hopkins Hosp. **32**:206 (June) 1921.

17. Bechold, H.: Colloids in Biology and Medicine, Trans. by J. G. M. Bullowa, New York, D. Van Nostrand Company, 1919.

of the surrounding medium. For though these vitally staining constituents may not be a part of the living cytoplasm, yet they are contained in it, and any substance which penetrates the cell membrane and changes their p_H must first pass through the cytoplasm.

CONCLUSIONS

1. The formed elements of the human blood are freely permeable to carbon dioxid and ammonium hydroxid.

2. The intracellular structures of living white blood corpuscles and blood platelets which stain vitally with neutral red may be made acid, alkaline, or remain essentially neutral by surrounding solutions with a p_H of 7.4.

3. These same solutions cause the reaction of the stained intracellular structures of dead white blood cells and the reticulum of erythrocytes to be the reverse of that produced in the living leukocytes.

4. Changes in the reaction within the cells are accompanied by opposite changes in the surrounding medium.

THE INFLUENCE OF FOOD INTAKE ON THE ENZYMATIC CONCENTRATION OF HUMAN INTESTINAL CONTENTS OBTAINED FROM A DUODENAL FISTULA *

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The rapidly increasing use of the duodenal tube has also greatly increased the need of practical chemical methods for the examination of duodenal contents, and as a result several systems have been described.[1] Of these methods, we have had most experience with the technic of McClure, Wetmore and Reynolds,[1] which was selected for this purpose because it appeared to us to be based on methods which had been carefully and scientifically tested out in other lines of work.

Two points operate against the practical application of the methods of examination of duodenal contents described by McClure and his collaborators. One of these is that the reagents used in the preparation of the substrate either are difficult to secure or are of varying composition. These authors recommend for this purpose an emulsion of cottonseed oil prepared by the Walker-Gordon Company of Boston, a preparation of paracasein (the soluble casein of Van Slyke and Bosworth) which, as far as we have been able to learn, cannot be bought on the market, but must be prepared in the laboratory, and soluble starch, which must be selected with the view of obtaining a preparation containing a minimum amount of reducing bodies. We have used throughout our work an emulsion of cottonseed oil secured from the Walker-Gordon Company, but have replaced the soluble casein (paracasein) recommended by a high grade of casein (Pfundstiehl's highest purity) and the soluble starch by the ordinary corn starch prepared by boiling 2 gm. of this substance with 100 c.c. of distilled water for half an hour; at the end of this period the starch paste is cooled, made up to the original volume (100 c.c.) and mixed with 100 c.c. of McClure's 0.2 molar phosphate mixture of p_H 8.4.

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* From the Department of Medicine and the Laboratory of Physiologic Chemistry of the Tulane University of Louisiana School of Medicine.

1. McClure, C. W.; Wetmore, A. S., and Reynolds, L.: New Methods for Estimating Enzymatic Activity of Duodenal Contents of Normal Man, Arch. Int. Med. **27**:706 (June) 1921.

The second objection to the practical application of duodenal analysis is the fact that McClure [2] recommends the use of a cream meal (50 c.c. of 20 per cent. cream) to be given by mouth after the duodenal tube has been passed. This procedure is often difficult of application as cream is frequently not available in hospitals except on special order, and its administration adds another rather troublesome detail even in office practice.

The use of the cream meal was recommended because it was found that the ingestion of fat apparently caused the flow of a juice richer in enzymes than that obtained after the use of carbohydrate or protein and it was believed by McClure and his collaborators that the examination of such a secretion, representing a fluid of maximum digestive power for the individual under examination, was of greater diagnostic value than the analysis of the secretion obtained from the fasting subject.

We have recently had the opportunity of collecting and examining a considerable number of specimens of duodenal juice from a patient with a duodenal fistula, from whom it was possible to obtain liberal portions of the duodenal contents by means of a syringe, the tip of which could be inserted through the fistulous opening. In view of the scarcity of published data regarding the enzymatic concentration of normal duodenal contents, we have felt that the publication of our results might furnish material of interest to workers in this field.

REPORT OF CASE

The patient, a negro laborer, aged 23, was an inmate of the New Orleans Charity Hospital, to which institution he had been admitted, Dec. 21, 1923, suffering from an injury caused by a pistol bullet which had entered the right side immediately below the costal border. On operation, which was performed by Dr. E. L. Irwin, a few hours after admission, repair was made of three perforations of the colon, and, as it was found that the projectile had made exit immediately posterior to the gallbladder, perforating this organ at its attachment to the liver, a cholecystostomy also was performed. After the operation, the patient made satisfactory progress for sixteen days, when, during a fit of coughing, the suture line gave way. In an emergency operation, the abdominal wound was again closed. Progress now continued to be satisfactory until Feb. 22, 1924, when it was noted that a fistulous opening had appeared in the upper third of the abdominal wound. Exploration of this fistulous opening, by Dr. Irwin, gave definite evidence of its duodenal origin.

After the third operation, the patient continued to make satisfactory progress, and, in spite of the persistence of the fistula, he gained rapidly in weight and strength. As judged by the amount and quality of the food consumed, he was apparently possessed of unusually good digestive powers, and as his cooperation was easily secured, he was found to be an excellent experimental subject.

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2. McClure, C. W., and Wetmore, A. S.: Studies in Pancreatic Function: Enzyme Concentration of Duodenal Contents After Ingestion of Pure Foodstuffs and Food Mixtures by Normal Man, Boston M. & S. J. **187**:882-895 (Dec. 14) 1922.

EXPERIMENTS

All experiments were performed in the forenoon when the patient was resting quietly in bed. As the secretion flowed more or less continuously from the fistula, it was necessary to have an assistant at hand to mop up the overflow with gauze. When a specimen was desired, the tip of an ordinary glass syringe to which had been attached a short piece of rubber tubing was inserted just within the opening of the fistula, and a sample sufficiently large for the determination of the enzymatic concentration and the p_H (by the electrometric method) was secured in a period of one to two minutes.

In every experiment, the patient was first observed in the fasting condition (from eighteen to nineteen hours after the last meal) and after a sufficient number of samples of fasting contents had been obtained, he was then fed by mouth definite amounts of relatively simple foodstuffs.

EXPERIMENT 1.—*Effect of Milk Ingestion on Duodenal Enzymes; Enzymatic Concentration*

Time, April 3, 1924	Amylolytic in Mg. Glucose	Lipolytic in C.c. Tenth Normal Sodium Hydroxid	Proteolytic in Mg. Nonprotein Nitrogen	p_H	Remarks
9:58 a.m.	2.6	0	4.6	6.86	Fasting about eighteen hours
10:00	2.2	0	4.6	6.66	Fasting
10:04	2.2	0	5	6.66	Fasting; 200 c.c. of milk given at 10:05, and 200 c.c. at 10:37
11:08	2.0	0.2	5.3	7.08	First curds appeared in fistula liquid at 10:46

EXPERIMENT 2.—*Effect of Cream Ingestion on Duodenal Enzymes; Enzymatic Concentration*

Time, April 6, 1924	Amylolytic in Mg. Glucose	Lipolytic in C.c. Tenth Normal Sodium Hydroxid	Proteolytic in Mg. Nonprotein Nitrogen	p_H	Remarks
10:22 a.m.	1.37	0.1	1.6	7.26	Fasting about nineteen hours
10:53	2	0	3.3	7.34	200 c.c. of 20 per cent. cream given at 10:27
11:13	1.4	1.1	3.3	7.5	A second 200 c.c. portion of cream given at 10:57
11:43	1.6	0.4	2.9	6.94	Curds from cream appeared in the fistula fluid at 10:58

EXPERIMENT 3.—*Effect of Egg Albumin Ingestion on Duodenal Enzymes; Enzymatic Concentration*

Time, April 8, 1924	Amylolytic in Mg. Glucose	Lipolytic in C.c. Tenth Normal Sodium Hydroxid	Proteolytic in Mg. Nonprotein Nitrogen	p_H	Remarks
9:25 a.m.	2.4	0	4.4	6.81	Fasting about eighteen hours
10:30	5	0	5.1	7.65	At 9:48, fed eight egg whites and one yolk boiled two minutes, and a small amount of sodium chlorid
11:05	5	1.5	8.7	6.59	Subject sleeping
11:26	2.8	1.3	7.6	6.42	Subject awake

EXPERIMENT 4.—*Effect of Starch Ingestion on Duodenal Enzymes; Enzymatic Concentration*

Time, April 17, 1924	Amylolytic in Mg. Glucose	Lipolytic in C.c. Tenth Normal Sodium Hydroxid	Proteolytic in Mg. Nonprotein Nitrogen	p_H	Remarks
9:50 a.m.	3.6	0.3	3.1	6.71	
10:14	5	0.1	5.7	7.17	Started eating 153 gm. of arrowroot biscuit
10:35	1.7	0.1	3.4	7.44	Finished crackers and drank 200 c.c. of water; at 10:33, crackers appeared in the secretion
10:53	2.26	0.2	6	7.45	Patient asleep
11:36	3	0.6	6.2	6.75	

SUMMARY

The influence on the pancreatic secretion of different foodstuffs taken by mouth has been demonstrated in the foregoing experiments; these observations were made under such conditions that there was no interference with the gastric function, which must necessarily come with the use of a duodenal tube.

The fasting duodenal fluid was collected and the enzyme concentration determined as a working basis in each experiment. The p_H of each specimen analyzed was always recorded.

The effect of ingesting a mixture of protein, fat and carbohydrate, in the form of 400 c.c. of milk, taken in two equal portions one-half hour apart, is a stimulation of the protease and lipase (Experiment 1). This increase in proteolytic and lipolytic activity was noted one-half hour after the subject took the last portion of milk.

The ingestion of fat in the form of cream, in two portions of 200 c.c. each, one-half hour apart, brought about a very distinct stimulation of the amylase and the protease within twenty-six minutes after taking the first portion. About twenty minutes later (sixteen minutes after the second portion of cream), there was noted a marked increase in the lipase concentration.

The ingestion of protein and a very small amount of fat, in the form of eight egg whites and one yolk boiled two minutes, was productive of the most striking results as yet observed. All three of the pancreatic enzymes assumed the maximum concentrations noted in any of the experiments made on this subject. In one hour and seventeen minutes after the eggs were taken, both the amylase and the protease had almost doubled their concentrations, whereas the lipolytic activity rose from 0 to 1.5 c.c.

The ingestion of the starch in the form of arrowroot biscuits, which proved very appetizing to the patient, did not, in spite of the psychologic effect, show any stimulation on the production of amylase and but little more on the protease.

CONCLUSIONS

Finally, it may be said that it is not our desire to draw any general conclusions from these experiments; however, the fact that this case offered an exceptional opportunity for studies of the enzymatic activity of the duodenal contents, collected without the possibility of disturbed normal function produced by the presence of a duodenal tube, accounts for our report of results that are very suggestive.

THE ELECTROCARDIOGRAM AS AN AID IN THE DIAGNOSIS OF ADHESIVE PERICARDIAL MEDIASTINITIS *

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Since the early days of the application of the string galvanometer to the study of the human heart, it has been known that in normal persons, on changing the position of the subject,[1] there is a change in the form of the record as secured in the three leads. This is especially prominent in patients with cardiac disease. It is best seen on turning the individual in the reclining position from one side to the other. It was thought that this phenomenon might be of practical use in the diagnosis of adhesive pericardial mediastinitis, of which we have no thoroughly reliable clinical sign. Normal persons and patients have been examined from time to time during the last three years to test this possibility.

The change in the form of the electrocardiogram affects all of the waves present to a greater or lesser degree, but is most easily seen in the Q-R-S group. All three leads are affected, but usually only two leads show marked alteration, and the third may remain to casual inspection essentially the same. Those which show conspicuous varition are normally Leads I and III. In electrodiograms of abnormalities, other combinations of leads may show the prominent alterations, depending on the position of the electrical axis.[2] For example, with so-called right ventricular preponderance (which might better be called predominance of the dextrogram), Leads I and II present the marked deviations.

The extent of the shift may be calculated mathematically in terms of the angle α of the electrical axis.[3] For this purpose, at least two, and preferably three, leads must be taken synchronously, with the proper precautions.[4] The values of the angle α are then determined for any selected instant in the electrocardiographic records secured in the two positions. As far as possible, the same instant in the cardiac cycle should be used in comparing records taken of an individual in different

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* From the cardiographic laboratory of the Johns Hopkins Hospital and University Medical Department.

1. Einthoven, W.: Lancet **1**:853, 1912.

2. Carter, E. P., and Dieuaide, F. R.: Bull. Johns Hopkins Hosp. **32**:219 (July) 1921.

3. Dieuaide, F. R.: Determination and Significance of Electrical Axis of Human Heart, Arch. Int. Med. **27**:558 (May) 1921.

4. Einthoven, W.; Bergansius, F. L., and Bijtel, J.: Arch. f. d. ges. Physiol. **164**:167, 1916. Cohn, A. E.: Heart **9**:311, 1922.

positions or at different times. It is not necessarily the case that the peak of R, for example, in a given lead occurs at the same instant under these circumstances, especially in electrocardiograms of diseased hearts. Hence, the use of such a criterion may give data which should not be compared. The best basis for comparing electrical axes is to take a certain instant after the earliest Q-R-S deviation in whatever lead that may occur.

Because of the relatively small number of cases which have been available for study, we have not thought it desirable to attempt to fix

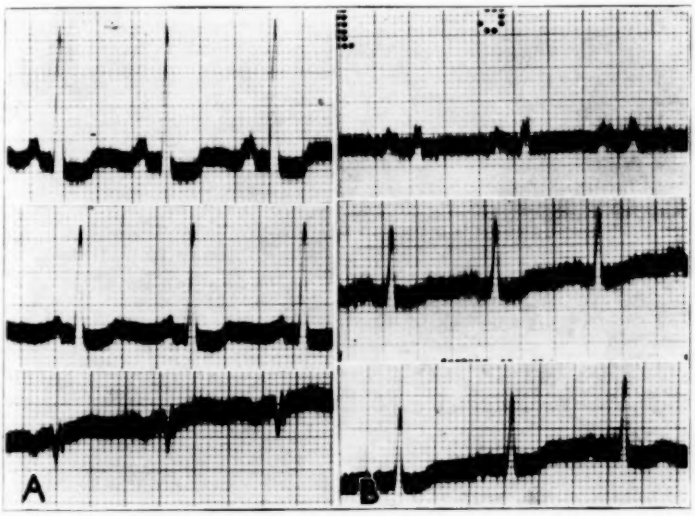

Fig. 1 (Case 2).—*A*, patient on right side; *B*, on left side; marked change in form of Leads I and III.

limits for the normal shift on changing the position of the subject, nor have we consistently determined the exact degree of shift in the pathologic cases, since the use of the mathematical determination of the electrical axis would greatly limit the availability of the procedure. We have, therefore, confined our interest to the cases in which, on careful inspection, there is practically no change in the form of the electrocardiogram.

In most instances, our procedure has been to take the three leads with the patient lying well over on his right side; then to turn the patient so that he lies on his left side and repeat the process. In many cases, respiration has such a marked effect on the form of the waves that a record of the respiratory phases should be made syn-

chronously. This may be readily done by means of the ordinary pneumograph with a tambour and lever set before the camera or by the spirometer method of Cohn.[4] Where it was necessary, we have used the pneumograph. Standardization of the galvanometer string should be carefully carried out and indicated on the record. If the standardization is not exact in the various leads and positions, the measurements may be corrected by appropriate calculations.

RESULTS

The significant results of this study are given in Table 1, which includes illustrative cases with shift of the electrocardiogram with

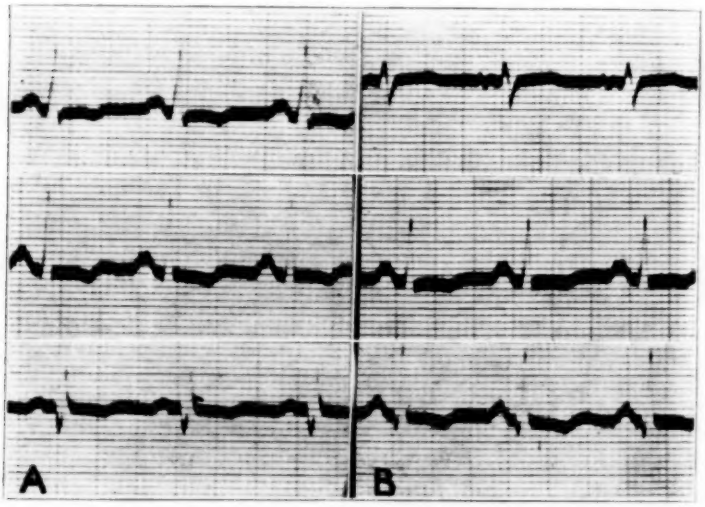

Fig. 2 (Case 3).—*A*, patient on right side; *B*, on left side; marked change in form of Leads I and III.

postmortem examination, and all of the cases, with or without necropsy, in which the record showed practically no change. Table 2 summarizes the data of all the observations.

No normal individuals in whom there was not a definite change in the form of the electrocardiographic record on rotation of the body have been encountered, although the change is sometimes not as marked as it is in patients with cardiac disease. In a series of fifty observations on patients with heart disease, four instances in which the record showed practically no shift were seen, and seven in which there was only a very slight shift. Thirty-three patients, including all those whose electro-

TABLE 1.—*Data of Illustrative Cases with Shift of the Electrocardiogram on Change of Position and All Cases with Insignificant Shift*

Case	Age	Type of Heart Disease	Clinical Signs of "Adhesive Pericarditis"	Electrocardiogram	Necropsy
1	39	Rheumatic	Apex fixed; systolic apical retraction	Marked shift	Pericardium, no lesion; mediastinum, no lesion; weight of heart, 740 gm.
2	48	Sclerotic coronary occlusion	Apex fixed	Marked shift Figure 1	Pericardium, no lesion; mediastinum, no lesion; weight of heart, 520 gm.
3	37	Hypertensive (septicemia Streptococcus hemolyticus)	Apex fixed	Marked shift Figure 2	Epicardium and pericardium densely adherent; mediastinum, no lesion; weight of heart and pericardium, 1,240 gm.
4	19	Rheumatic	Apex fixed; systolic apical retraction	Marked shift Figure 3	Pericardium, no lesion; mediastinum, no lesion; weight of heart, 600 gm.
5	38	Syphilitic	(1) (Pericardial rub) (2) Apex fixed; Broadbent's sign	(1) Slight shift (2) No shift; Figure 4	Epicardium and pericardium densely adherent; many adhesions between mediastinum and pericardium; weight of heart, 980 gm.
6	21	Rheumatic	(Pericardial rub); apex fixed	No shift Figure 5	Pericardial cavity obliterated; dense pleuropericardial adhesions; weight of heart, 1,050 gm.
7	21	Rheumatic	(Pericardial rub); apex fixed; systolic apical retraction	Slight shift Figure 6	Pericardial cavity obliterated; delicate pleuropericardial adhesions; weight of heart, 800 gm.
8	36	Syphilitic	Apex fixed; systolic apical retraction; diastolic shock	Slight shift	Moderate adhesions between epicardium and pericardium; many pleuropericardial adhesions; weight of heart, 850 gm.
9	45	Nephritic	(Pericardial rub); apex fixed; systolic apical retraction	No shift	
10	22	Rheumatic	Apex fixed; systolic apical retraction; Broadbent's sign	Slight shift	
11	16	Rheumatic	Apex fixed; Broadbent's sign	Slight shift	
12	23	Syphilitic; tuberculous	Apex fixed	Slight shift	
13	22	Tuberculous	(Pericardial rub); apex fixed; diastolic shock	Slight shift	
14	15	Rheumatic	Apex fixed; (pericardial rub?)	Slight shift	
15	44	Rheumatic?	(1) (Pericardial rub) (2) Apex fixed; systolic apical retraction	(1) Marked shift (2) No shift	

(1) Early admission; (2) last admission.

TABLE 2.—*Summary of Results*

Total observations		50
Cases with signs of "adherent pericardium"		33
No shift of electrocardiogram (all with clinical signs)		4
Necropsies	2	
(Both showing dense adhesions of pericardial cavity and mediastinum)		
Very slight shift of electrocardiogram (all with clinical signs)		7
Necropsies	2	
(Both with moderate involvement of pericardium and mediastinum)		
Marked shift of electrocardiogram (all with clinical signs)		22
Necropsies	8	
(None with combined pericardial and mediastinal lesions)		

cardiogram did not change significantly, had some indication of signs which are frequently associated with "adherent pericardium." Twelve patients came to necropsy, including eight with signs suggestive of the lesion and, among these eight, four patients whose electrical axis remained relatively constant. All four of these individuals with fixed electrical axes had important lesions of the pericardium and of the mediastinum which were present in none of the other patients on whom postmortem examinations were done.

In Case 3, although the pericardial cavity was practically obliterated, there were no lesions of the mediastinum. In Case 4, there were a

Fig. 3 (Case 4).—*A*, patient on right side; *B*, on left side; marked change in Leads I and II.

few delicate adhesions between the mediastinum and the pericardium, but none within the pericardium. The records of both these patients changed markedly with the rotation of the individuals. In Cases 7 and 8, the lesions of the mediastinum were not marked, and the electrocardiograms shifted slightly.

It is noteworthy that the series includes several patients with marked enlargement of the heart (in one case the heart weighed 860 gm.), whose records showed the usual shift (Figs. 1, 2 and 3).

Of especial interest, at least potentially, is the case of the patient (Case 15) who when first seen had an attack of acute pericarditis, at which time the electrocardiogram changed with the change in position.

Two months later, there were suggestive clinical signs of adhesive pericardial mediastinitis, and the record remained constant. This patient is still under observation.

COMMENT

Of the difficulty of making the diagnosis of adhesive pericardial mediastinitis, little need be said. It has long been recognized that adhesions between the two pericardial layers, although they may lead

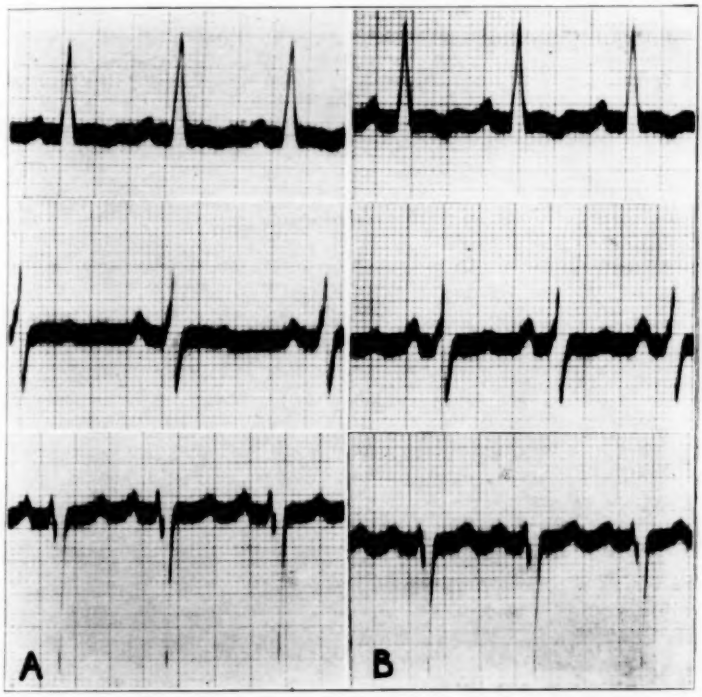

Fig. 4 (Case 5).—*A*, patient on right side; *B*, on left side; no change with shift in position.

to embarrassment of the heart, can hardly be diagnosed clinically. On the other hand, mediastinitis, perhaps as a rule, does not involve the heart at all. Therefore, the condition which we may hope to diagnose clinically, and which is of great importance from the cardiac standpoint, is a combination of chronic adhesive pericarditis, properly speaking, and mediastinitis. This may be termed chronic adhesive pericardial mediastinitis. It is to this condition that the sign here reported, if it is confirmed, has reference.

The normal change in the electrocardiogram is, of course, undoubtedly due to movement of the heart and the consequent bringing into the record of potential differences in planes which are not recorded in the usual position. That it is not due entirely to lateral movement is shown by the fact that it may occur when the apex is fixed, and also by the fact that changes in the position of the heart produced by pneumothorax or pleural effusion have only a small influence on the form of the record. (It is to be remembered that the effect in the cases

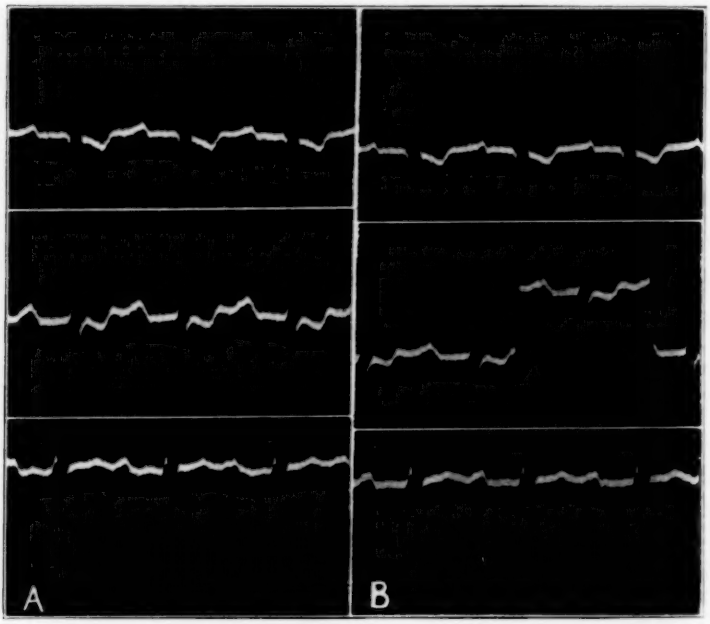

Fig. 5 (Case 6).—*A*, patient on right side; *B*, on left side; no change with shift in position.

of true dextrocardia is due to reversal of the chambers of the heart, together with the branches of the bundle of His.) The change is, therefore, due to rotation of the heart about a longitudinal axis. It might be expected to disappear when the heart is fixed, since there can then be no change in the relation of the plane from which the potential differences are recorded and the ultimate leading off points, which are, of course, not the extremities.

In some instances, the interpretation of the records secured is difficult. This is not infrequently the case in the presence of auricular

fibrillation, owing to intrinsic variation in the amplitude of all of the waves. Figure 3 shows, however, that the shift may be readily seen in some cases. In the absence of auricular fibrillation, the same phenomenon may rarely cause difficulty. In one case, we have obtained within a short time of each other records showing a moderate change and practical fixation of the electrical axis. No satisfactory explanation can be offered for this, although, on the basis of the clinical evidence, the impression is that fixation of the heart is in progress.

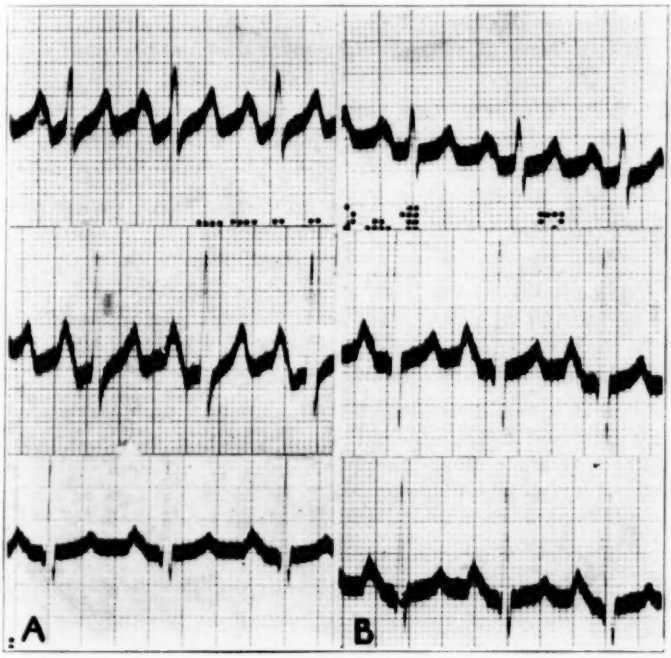

Fig. 6 (Case 7).—*A*, patient on right side; *B*, on left side; slight change with shift in position.

The evidence available does not warrant an attempt to estimate partial fixation of the heart; nor should this be done without suitable anatomic control.

Theoretically, the phenomenon reported may be expected to occur. The number of cases available for complete study has been small and is, perhaps, not conclusive evidence. It is significant, however, that failure of the electrocardiogram to change with the shift in the position of the subject is unusual and apparently of no greater frequency than

fixation of the heart as a postmortem finding. It is hoped that evidence may be accumulated, and it is suggested that patients with acute pericarditis who may later develop the chronic lesions, as well as those suspected of having chronic adhesive pericardial mediastinitis, be studied in the manner described.

SUMMARY

1. Normally there is a change in the form and amplitude of the electrocardiographic waves on shifting the subject from the right to the left side. This phenomenon is marked in most patients with heart disease. It is due to rotation of the heart.

2. It is shown that cardiac enlargement does not prevent the usual change.

3. Clinically, we may only hope to diagnose instances of "adherent pericardium" in which both the pericardial cavity and the mediastinum are involved by adhesions.

4. A small series of patients is reported who showed no significant change in their electrocardiographic record with a shift in position. All of these who came to necropsy were found to have important lesions involving both the pericardium and the mediastinum. Of a larger series of patients with clinical signs of "adherent pericardium," whose records showed a marked change, none were found to have lesions both of the pericardium and of the mediastinum.

5. It is suggested that fixation of the electrical axis, determined by this means, may serve as objective evidence of this lesion.

THE RESPIRATORY GAS PERCENTAGES DURING NITROUS OXID ANESTHESIA IN DOGS*

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The practical induction of nitrous oxid-oxygen anesthesia by the methods now universally practiced still leaves undetermined the question of adequacy of the supply of oxygen to the patient.

The question as to availability of oxygen is rendered the more important by recent work which tends to establish the minimum requirements of oxygen in respiratory airs for efficiency in the normal individual. The experimental data of the United States Army School of Aviation Medicine prove that a normal man cannot long retain consciousness unless he breathes air of at least 6 per cent. oxygen.[1] In the tests, it is assumed that the individual is exerting a minimum of muscular activity. He is not far from the normal basal metabolic level. By the least additional work, as by any sudden muscular acts, he quickly consumes the oxygen below the level necessary to retain consciousness. For less resistant individuals, either normal or pathologic, a greater percentage of oxygen must be supplied. This critical oxygen level in the Air Service tests is not far different from that of the oxygen percentages in nitrous oxid-oxygen mixtures during practical anesthesia.

Working with dogs, we have undertaken to study the percentage of the gas mixtures which these animals must breathe to induce a surgical stage of anesthesia, namely, complete muscular relaxation and the regular respirations and circulatory conditions characteristic of an even stage of anesthesia.

REVIEW OF LITERATURE

Not much has been done in the analysis of the respiratory gases during prolonged nitrous oxid-oxygen anesthesia. Kemp,[2] in 1896,

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*The data of this paper were presented in a thesis by the junior author in partial fulfilment of the requirements for the degree of Master of Arts in the University of Missouri.

*We are indebted to the Committee on Therapeutic Research of the American Medical Association for aid in the purchase of necessary apparatus.

1. The literature on anoxemia is listed by Schneider, E. C., and Truesdell, Dorothy: Am. J. Physiol. **55**:223 (March) 1921; and by Greene, C. W., and Gilbert, N. C.: Am. J. Physiol. **60**:155 (March) 1922.

2. Kemp, G. T.: Nitrous Oxid Anesthesia, Brit. M. J. **2**:1480, 1897.

supplied his experimental dogs with nitrous oxid and air, the percentages not given, and made five experiments with 10, 6.1, 6.5 and 11.5 per cent. oxygen in the mixtures breathed. It is not clear from his data what stage of anesthesia was reached, especially in the experiments with the higher percentages of oxygen with nitrous oxid.

Cullen, Austin, Kornblum and Robinson [3] published the results of two experiments in which they state that "it was found impossible to secure anesthesia in the dog without using 95 per cent. or more of nitrous oxid. This inevitably led to marked anoxemia." These observations were made in their study of the initial acidosis in anesthesia. They used pure oxygen, 5 parts, with nitrous oxid, 95 parts. They also tested anoxemia by administering nitrogen followed by a mixture of 95 parts nitrogen and 5 parts oxygen.

Leake and Hertzman,[4] in studies on blood reaction in ethylene and nitrous oxid anesthesia, administered nitrous oxid and oxygen to dogs using 85, 90 and 95 per cent. by volume of nitrous oxid with pure oxygen in their inhalant mixtures. They state: "Under nitrous oxid and oxygen, it is impossible to obtain true anesthesia without some degree of anoxemia."

EXPERIMENTAL METHODS

In the present experiments, normal dogs were anesthetized through a face mask. They were given pure nitrous oxid for a certain number of respirations in order to remove the excess of pulmonary and tissue nitrogen, then switched to a predetermined mixture of nitrous oxid and oxygen. The mixture was conducted to the face mask through one-way flutter valves. The mixing was obtained in a large gasometer, and by a McKesson nitrous oxid-oxygen apparatus. The exhalant tube was so controlled as to discharge into a rebreather, into outside air, or into a Tissot apparatus, thus enabling one to examine the exhaled as well as the inhaled gases.

Dogs vary in their susceptibility to nitrous oxid in oxygen mixtures. Broadly speaking, by this technic it requires a reduction to 6 and sometimes to 5 per cent. of oxygen in the inhaled gas to induce and maintain anesthesia. Very slight variations from the necessary concentration for the particular animal would either induce light anesthesia or stop respirations, according to the direction of the change in mixture. The narrow working margin was always a surprise.

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3. Cullen, G. E.; Austin, J. H.; Kornblum, K., and Robinson, H. W.: The Initial Acidosis in Anesthesia, J. Biol. Chem. **56**:679 (June) 1923.

4. Leake, C. D., and Hertzman, A. B.: Blood Reaction in Ethylene and Nitrous Oxid Anesthesia, J. A. M. A. **82**:1162 (April 12) 1924.

Gas samples were taken from this apparatus through three channels: (1) from the inhalant tube between the McKesson mixer and the respiratory valves; (2) from the exhalant tube outside the valve system; (3) in a certain number of experiments, deep alveolar gas samples were taken. From analyses of the first, the accuracy of the mixture inhaled was confirmed, and from the other samples the various data as to respiratory oxygen availability and carbon dioxid output were obtained.

GAS ANALYSIS METHODS

The Haldane air analysis apparatus was used for gas analysis, the principal steps in the analysis being, first, the absorption of carbon dioxid by caustic potash; second, the absorption of oxygen by an alkaline pyrogallate solution, and third, the estimation of nitrous oxid by difference. The method of analysis is applicable to nitrous oxid-oxygen-carbon dioxid mixtures without nitrogen, or with known nitrogen. In this series, the samples were taken only after the respiration had proceeded to a point where it could be safely assumed that all the nitrogen or at least all but mere traces had been removed.

Nitrous oxid, like carbon dioxid, is extraordinarily soluble.[5] Kunerth has shown that nitrous oxid is soluble in water to as much as 44.9 per cent. by volume at 36 C. It is sparingly soluble in saturated potassium hydrate solutions and in saturated potassium hydrate-pyrogallate solutions. However, the physical solubility of the gas must be taken into account in determining the total nitrous oxid in a gas sample by this method.

Solubility curves were run on the apparatus used and on mixtures containing known quantities of nitrous oxid and oxygen. From these, a correction factor was obtained for the approximate mixtures found in actual analyses. The percentage of error by this technic is large for physical purposes, but it is insignificant in view of the main question at issue.

EXPERIMENTAL RESULTS

Over fifty experimental gas analyses were made in order to determine the range of oxygen-nitrous oxid mixtures, during effective anesthesia. In the dog, it is difficult to identify comparable states of anesthesia. Deep anesthesia is characterized by complete muscular relaxation. However, loss of voluntary motion occurs much earlier, but muscular clonic contractions may occur up to the onset of deep

5. Kunerth, William: The Solubility of Carbon Dioxid and Nitrous Oxid in Certain Solvents, Physical Review **19**:512, 1922.

anesthesia. Dogs will give uncoordinated vocalization at a concentration of nitrous oxid within 1 per cent. of an effective anesthetic mixture. For example, an animal in surgical anesthesia at 6 per cent. oxygen and 94 per cent. nitrous oxid will soon recover sufficiently from this stage to give uncoordinated vocalization when the percentage of oxygen is raised to 7 with 93 per cent. nitrous oxid. During experiments, the respiratory movements were recorded by the Sargents belt stethograph. The character of the respiratory tracing furnished a very good supplementary index of the stage of anesthesia.

The experiments are set forth in groups, depending somewhat on the development of technic during the work.

TABLE 1.—*Analysis of Initial Mixture and Residual Gas in Apparatus After Complete Relaxation*

				Initial Rebreather Mixture			At Establishment of Anesthesia		
Experiment	Date	Dog	Temperature	Oxygen, per Cent.	Nitrous Oxid, per Cent.	Carbon Dioxid, per Cent.	Oxygen, per Cent.	Nitrous Oxid, per Cent.	Carbon Dioxid, per Cent.
1	1/31	1	24	12.31	87.67	0.02	3.5	95.37	0.213
2	2/ 2	1	24	9.82	91.08	0.1	2.28	98.4	0.458
3	2/ 2	2	24	11.75	87.97	0.25	2.17	95.64	0.219
4	2/ 7	2	25	11.64	88.36	0	2.91	97.08	0
6	2/ 7	2	25	3.29	96.7	0	3.11	96.89	0
7	2/ 7	2	25	4.67	95.33	0.07	3.96	96.01	0.03
9	2/ 9	1	26	4.81	95.17	0.02	4.84	95.16	0.01

Nitrous oxid-oxygen mixtures were given from a rebreather in which the first few exhalations were wasted to the exterior, then the system closed. The gas samples at the close of the experiment were taken from the exhalant tube before carbon dioxid absorption, in Experiments 1 to 3; from the inhalant tube just outside the valves, in Experiments 4 to 9; hence these represent the composition of the gases inhaled and exhaled at the moment anesthesia was established.

In Group 1, a large volume of an initial mixture assumed to be adequate for anesthesia was rebreathed by the animal until anesthesia was induced. Analyses of the initial mixture and of the residual gas in the apparatus after complete relaxation are presented.

In Group 2, the animal also rebreathed a mixture by the closed circuit method. But the animal was given a corresponding preliminary gas-oxygen mixture from the McKesson apparatus for a sufficient time to remove the nitrogen from the lungs and system. It was then switched to the rebreather filled by the same mixture but checked by analysis. The terminal gases were also analyzed, in this group. The advantage of the method consists in the fact that the oxygen was progressively but slowly reduced in the mixture, thus enabling one to judge more accurately the partial percentages of nitrous oxid at the different stages of anesthesia as between light, medium and deep.

TABLE 2.—*Second Group Given Preliminary Gas-Oxygen Mixture from McKesson Apparatus*

Experiment	Date	Dog	Temperature	Initial Rebreather Mixture: Oxygen, per Cent.	Initial Rebreather Mixture: Nitrous Oxid, per Cent.	Initial Rebreather Mixture: Carbon Dioxid, per Cent.	At Establishment of Anesthesia: Oxygen, per Cent.	At Establishment of Anesthesia: Nitrous Oxid, per Cent.	At Establishment of Anesthesia: Carbon Dioxid, per Cent.	
10	2/ 9	1	24	—	—	0	3.65	96.35	0	Deep
11	2/14	2	24	4.77	95.23	0	3.77	96.23	0	Medium
12	2/14	2	24	7.87	92.13	—	3.82	96.18	—	Medium
13	2/15	2	24	7.61	92.39	0.08	4.19	95.79	0	Light
14	2/15	2	25	—	—	0	4.19	95.79	0	Medium
15	2/16	2	25	4.92	95.08	0	3.88	96.12	0	Deep
17	2/23	2	23	7.62	92.35	0.03	3.62	96.38	0.025	Light (?)
18	2/23	2	24	4.64	95.36	—	3.8	96.2	0	Light (?)
19	2/28	3	24	4.25	95.75	—	4.24	95.75	0.015	Medium
20	2/28	3	24	5.5	94.48	0	4.04	95.96	0	
22	3/ 2	3	24	4.75	95.25	0	4.15	95.85	0	Medium

Nitrous oxid-oxygen mixtures were given from a closed rebreather. This set differs from Group 1, Table 1, in the thoroughness of the preliminary washing out of nitrogen from the lungs and tissues by gas mixtures from a McKesson apparatus before connecting with the rebreather mixture.

Group 3 received nitrous oxid-oxygen directly from the McKesson apparatus, wasting the exhaled gases into the external air, with samples into a Tissot apparatus during the anesthesia stages desired. This technic closely imitates the practical methods of gas administration in that the gas mixture can be varied instantly, or the animal can be held for long periods at a definite and constant stage of anesthesia. Respired mixtures were sampled from the inhalant and exhalant tubes outside the controlling valves.

TABLE 3.—*Third Group in Which Gas Mixtures Were Supplied Directly from McKesson Apparatus*

Experiment	Date	Dog	Temperature	Inhaled Gases: Oxygen, per Cent.	Inhaled Gases: Nitrous Oxid, per Cent.	Inhaled Gases: Carbon Dioxid, per Cent.	Exhaled Gases: Oxygen, per Cent.	Exhaled Gases: Nitrous Oxid, per Cent.	Exhaled Gases: Carbon Dioxid, per Cent.	
23	3/ 7	3	25	21.23	79.75	0.02	—	—	—	
24	3/ 7	1	24	5.71	94.29	0	—	—	—	
25	3/ 9	4	23	2.37	97.5	0.13	—	—	—	
26	3/ 9	4	25	3	97	0	—	—	—	Light
27	3/14	4	23	6.86	93.14	0	—	—	—	Light
28	3/14	3	26	5.63	94.37	—	—	—	—	Light
29	3/16	4	23	7.48	92.34	0.18	5.93	92.58	1.49	Light
30	3/16	3	23	6.54	93.46	—	5.43	92.75	1.82	Light
31	3/21	3	26	6.72	93.2	0.08	5.21	93.44	1.45	Medium
32	3/23	4	23	5.09	94.91	0	4.38	93.6	2.02	Light
33	3/28	3	24	6.74	93.26	0	—	—	1.37	Light
34	3/28	4	24	11.49	85.51	0	9.41	88.32	2.27	Very light
35	3/30	4	24	6.71	93.29	0	5.44	92.93	1.63	Light
36a	4/ 4	3	22	4.06	95.94	0	3.86	94.93	1.32	Medium
36b	4/ 4	3	23	3.54	96.46	0	—	—	—	
37	4/ 6	4	23	10.75	85.25	0.12	8.47	89.37	2.16	Light
39	5/ 2	3	24	7.85	92.15	0.08	6.21	91.63	1.86	
40	5/ 4	3	21	9.04	90.96	0	8.01	89.27	2.11	
41	5/ 4	3	23	6.18	93.82	0	5.34	95.51	1.44	Light
42	5/ 4	3	26	4.4	95.60	0	2.9	95.24	1.77	Deep
43	5/ 4	3	23	9.6	91.4	0	—	—	—	Light
44	5/ 8	4	22	14.66	85.44	0	11.08	85.44	3.48	Very light ?
45	5/ 9	5	23	7.64	92.36	0	—	—	—	Light
46	5/ 9	5	24	3.57	96.43	0	—	—	—	

Samples of inhalant and exhalant gases were simultaneously drawn from outside the corresponding valves at the stage when anesthesia was constant.

In Group 4, chlorbutanol was administered to keep the animal quiet on a chlorbutanol metabolism level. The relation of the inhaled and exhaled gases is shown. But this series was done primarily to cooperate with simultaneous blood gas studies.

In these series, the records of respiratory movements were invaluable as a supplement to direct observations of the animal itself. In the first series, the analytic samples were taken during the more profound stages of anesthesia. The percentage of oxygen, at this time, was unexpectedly low, between 2.17 and 4.84 per cent. The nitrous oxid averaged, therefore, about 95 per cent. in these mixtures. Compared with man, the dog is known to be much more resistant to low oxygen. In a long series of experiments on anoxemia, Greene and Gilbert [1] have shown that dogs do not cease breathing until the amount of oxygen available in the air they breathe is reduced to between 3 and 4 per cent. They report one experiment with as low as 1.96 per cent. of alveolar

TABLE 4.—*Fourth Group in Which Chlorbutanol Was Administered*

				Inhaled Gases			Exhaled Gases		
Experiment	Date	Dog	Temperature	Oxygen, per Cent.	Nitrous Oxid, per Cent.	Carbon Dioxid, per Cent.	Oxygen, per Cent.	Nitrous Oxid, per Cent.	Carbon Dioxid, per Cent.
47	5/11	6	24	22.72	77.28	0.02	21.28	77.32	1.4
48	5/11	6	22	20.18	79.82	0	19	79.28	1.72
49	5/11	6	24	7.31	92.66	0	7.7	91.4	0.97
50	5/18	7	20	17.29	82.71	0	11.51	83.09	5.4
51	5/18	7	23	7.83	92.17	0	4.51	91.79	3.74
52	5/18	7	24	17.75	82.25	0	14.32	84.38	4.12
53	5/18	7	24	8.83	91.17	0.16	5.88	90.87	3.25

These data were obtained from chloretonized dogs manipulated as in Group 2, except that rebreather records were taken to determine the rate of oxygen consumption. Simultaneous inhalant and exhalant gases (before carbon dioxid absorption) are presented.

oxygen at the moment respirations stopped. The present nitrous oxid-oxygen series approaches in concentration of nitrous oxid that observed for nitrogen anoxemia in their experiments.

When the inhaled gas from a rebreather is analyzed at the earliest identifiable surgical anesthesia stage, the values for oxygen have been from 4.9 to a minimum of 3.6 per cent., figures somewhat higher than in the first series.

In Group 3, consisting of twenty-one experiments, the nitrous oxid-oxygen mixture was supplied directly from the mechanical mixing valves of the McKesson apparatus. Samples of both inhalant and exhalant mixtures were analyzed, and attempts were made to secure a variety of experimental stages from very light to deep anesthesia. The stages were judged in a large degree by the response to sensory stimulation, by the degree of muscular relaxation, and by the amount of reflex or voluntary control. In the dog, the eye reflexes are of comparatively

little value in determining stages of nitrous oxid narcosis, since the eye reflex is retained even in the deepest stages of anesthesia.

In the lightest anesthesias, for example, Experiments 34, 37 and 40, the exhaled gas contained from 8.01 to 9.41 per cent. oxygen, an amount considerably above the inhaled percentages of oxygen during very deep anesthesias. These were not relaxed enough to be surgically satisfactory. It is questionable whether they should be classified as true anesthesias. Experiment 44 was even more questionable. In our series, very deep anesthesia was induced only by an inhalant gas mixture of less than 5 per cent. by volume of oxygen.

Attempts were made to secure samples of respiratory gases from an animal at successive stages during one experimental gassing. The results may be seen in Experiment 45, for light anesthesia, and Experiment 46, for very deep anesthesia. These two experiments illustrate the fact that the inhalant mixture, for light anesthesia, must contain not more than 7.6 per cent. by volume of oxygen, and, for deep anesthesia, as little as 3.49 per cent. by volume. The corresponding exhaled gases contained 5.19 and 2.58 per cent. by volume. This particular dog was somewhat less resistant to low oxygen than many observed, yet the outstanding facts indicate that in him nitrous oxid-oxygen anesthesia of the surgical stage can be successfully approached only by approximately from 94 to 96 per cent. by volume of nitrous oxid in the inhaled mixture. Such experiments lend support to the assertion of Dr. McKesson in his chairman's address before the American Association of Anesthetists at San Francisco, June 6, 1923, that nitrous oxid serves its primary function as a diluent of oxygen during the induction of nitrous oxid-oxygen anesthesia. So low a percentage of oxygen in inhaled airs inevitably induces a degree of anoxemia.

Throughout the progress of these experiments on dogs, there were general facts of outstanding import which occurred over and over again. Most important of all was the quick response of the animal to the slightest variations in the percentage mixture of nitrous oxid and oxygen from the anesthesia mixture. If the proportion of nitrous oxid was reduced only 1, or at most 2, per cent., dogs would recover within a few minutes; if increased, respirations would quickly slow down and stop. If respirations ceased, however, a single artificial respiration of pure air would usually suffice to restore the animal to normal breathing. In other series of experiments in which blood pressure was measured during nitrous oxid anesthesia, it was determined that the circulation was strong and vigorous at the time respirations ceased, a fact which coincides with the condition of the circulation in extreme anoxemia.

SUMMARY

1. Dogs, anesthetized by adequate mixtures of nitrous oxid and oxygen, pass through stages of analgesia, loss of reflexes and muscular relaxation. These stages cannot be produced by nitrous oxid except in the presence of a proportionate reduction of oxygen, ranging from a maximum of 11.5 per cent. down to a lower limit of 3.6 per cent. by volume. The higher oxygen percentages will not induce satisfactory surgical anesthesia in dogs.

2. During light and borderline anesthesia, the amount of oxygen in nitrous oxid-oxygen mixtures exhaled by the dog may be from 8.01 to 9.41 per cent., but averages lower, from 4.38 to 5.93 per cent.; in medium anesthesias, from 3.88 to 5.20 per cent. by volume. The lower figure induces profound relaxation.

3. Analyses of exhaled nitrous oxid-oxygen mixtures indicate that a residuum of from 3 to 4 per cent. by volume of oxygen is adequate to support the basal physiologic processes.

4. Complete and rapid recovery from nitrous oxid-oxygen anesthesia takes place in the dog within from thirty seconds to three minutes from the time the animal begins breathing atmospheric air.

5. Dogs will stand repeated administrations of nitrous oxid-oxygen through a period of months with no apparent ill effects to the animal.

THE DISTRIBUTION OF NITROUS OXID AND OXYGEN IN THE BLOOD OF DOGS DURING GAS ANESTHESIA *

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Nitrous oxid was discovered by Priestley [1] in 1774, and its anesthetic properties by Sir Humphrey Davy [2] in 1800. The first attempt to measure the amount of the gas in dog's blood during anesthesia was made by Jolyet and Blanche [3] in 1873, a full century after Priestley's discovery. Oliver and Garrett,[4] 1893, were the first to analyze all the gases of the blood of the dog, comparing the gas distribution before and during nitrous oxid anesthesia. Unfortunately, their results are subject to serious question, and have not been fully confirmed. In the meantime, thousands of nitrous oxid gas anesthesias were performed, and a large body of symptomatic data was developed before the determination of the scientific physiochemical facts on which their explanation rests. The delay was due in no small measure to the difficulties of method.

The ingenious and very workable micro-apparatus of Van Slyke,[5] 1914, for blood gas analysis has opened anew the field of blood gas studies, because of its far-reaching scientific and clinical applicability. Van Slyke and his collaborators have already published classic contributions. We have sought to apply the methods to a more detailed study of the facts of nitrous oxid distribution in the blood. The practice of mixing oxygen with the nitrous oxid to prolong anesthesia, and the ever increasing physiology of anoxemia both emphasize the vital importance of the availability of oxygen during gas anesthesia. These are the considerations which have inspired the development of the present work.

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* From the department of physiology and pharmacology, laboratory of pharmacology, University of Missouri School of Medicine.

* We are indebted to the Committee on Therapeutic Research of the American Medical Association for aid in the purchase of necessary apparatus.

1. Priestley, Joseph: Experiments and Observations on Different Kinds of Air; Memoirs of Joseph Priestley to the Year 1795, 1803.
2. Davy, Humphrey: Research, Chemical and Philosophical, Chiefly Concerning Nitrous Oxid, London, 1800.
3. Jolyet and Blanche: Compt. rend. de biol., June, 1873, p. 223.
4. Oliver and Garrett: Lancet **2**:625, 1893.
5. Van Slyke, D. D.: J. Biol. Chem. **30**:347 (June) 1917.

HISTORY

The history of nitrous oxid is brought up to the time that is of most importance to this paper by the four recorded dates of our introductory paragraph. To Andrews [6] of Chicago belongs the honor of adding oxygen to nitrous oxid to render it nonasphyxial. This was an epoch making step. It has proved to be the first practical act in refutation of the prevalent idea of Davy that the body could in some way split off and utilize the oxygen of the nitrous oxid molecule. Andrews made no analyses but rested his practice on the clinical evidence of improvement on adding free oxygen, a step for which, as Gwathmey rightly states, he scarcely received adequate credit at the time.

Bert,[7] 1878, also added oxygen to nitrous oxid and, in addition, put his patients under an increased pressure in a pneumatic chamber. Bert used this method to confirm argument in favor of the positive anesthetic property of the gas. From 80 to 85 per cent. nitrous oxid with from 15 to 20 per cent. oxygen under from 5 to 6 atmospheres

TABLE 1.—*Gases of Dog's Blood, Per Cent. by Volume (Oliver and Garrett)*

	Before Inhalation	After Inhalation
Oxygen	22	3.49
Carbon dioxid	34.3	15.66
Nitrogen	1.8	11.23
Nitrous oxid	....	22.49

of pressure anesthetized the patient, while the physician outside the chamber, with the corresponding percentages of nitrogen and oxygen of atmospheric air, was of course quite normal. The great solubility of nitrous oxid in the body tissues was not known, and not emphasized as a possible source of displacing oxygen until Hermann [8] called attention to it in support of the asphyxiation theory, in 1860.

The first effective attempt to determine the amount of gases soluble in the blood during anesthesia was made by Oliver and Garrett,[4] in 1893. They analyzed the blood of the dog. Their analyses are so significant that the figures are requoted in Table 1.

It is noteworthy that these authors found 1.8 per cent. by volume of nitrogen in normal dog's blood, a figure that was exactly confirmed by the far more carefully guarded determinations of Van Slyke. The excess of nitrogen which they found after gas inhalation was taken as an index of the amount of oxygen made available, following Davy's theory of dissociation. But it is now known that their 1.8 per cent. by

6. Andrews, E.: J. Brit. Dent. Soc. **22**, 1869.
7. Bert, Paul: Compt. rend. Acad. d. sc. **2**:87, 728, 1878.
8. Hermann: Brit. M. J., 1868, p. 378.

volume before anesthesia represents the saturation of arterial blood by nitrogen under the partial pressures in atmospheric air. Evidently, so great an amount as 11.23 per cent. of nitrogen, reported by Oliver and Garrett after nitrous oxid inhalation, represents an impossible supersaturation. The figures can only be interpreted as due to an error from some source not revealed.

Kemp [9] restudied the gases of dogs' blood to determine by actual measurements the old question of asphyxial (Hermann) versus anesthetic (Davy, Bert) properties of nitrous oxid. Kemp, like Bert, compared nitrous oxid-oxygen mixtures with corresponding nitrogen-oxygen mixtures, but he did not vary the barometric pressures as did Bert. Kemp used the cumbrous Hemple methods, fraught with the usual difficulties of extracting large samples of blood. His results in twelve experiments are briefly: Nitrous oxid of the blood varied from 6.9 to 33.5 per cent. by volume. There were only two determinations below 20 and more than half were above 28 per cent. by volume. Nitrogen averaged 0.4 per cent. by volume, with a maximum of 1.1 per cent. Oxygen was found to vary between 2.9 and 17.8 per cent. by volume. If all experiments are omitted from Kemp's table for which he records explanatory remarks for irregularities, it appears that the oxygen found falls between 7.9 and 14.2 per cent. by volume. He did not determine the oxygen capacities of his blood samples; hence, the percentage saturation cannot be calculated. It is important that Kemp did not confirm Oliver and Garrett's record of an excess of nitrogen in the blood in nitrous oxid anesthesia.

Nicloux,[10] 1908, in careful analyses of blood, found from 20 to 25 per cent. by volume of nitrous oxid during anesthesia, and reported 30 per cent. by volume at the onset of death. These figures are more constant and somewhat higher than those of Kemp.

Leake and Hertzman [11] have just published studies to determine the course of the p_H during prolonged anesthesia. They find that true anesthesia cannot be obtained without a degree of anoxemia. There is at first an increase followed later by a fall in the p_H, which points toward an uncompensated acidosis. The femoral blood contained an oxygen saturation of from 82.3 to 96.2 per cent. by volume, a degree of saturation considerably higher than the averages we have found for deep and surgically relaxed stages of anesthesia.

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9. Kemp, G. T.: Brit. M. J. **2**:1480, 1896. This article has an extensive reference list.

10. Nicloux, M.: Compt. rend. Soc. de biol. **1**:450, 1908.

11. Leake, C. D., and Hertzman, A. B.: Blood Reaction in Ethylene and Nitrous Oxid Anesthesia, J. A. M. A. **82**:1162 (April 12) 1924.

METHODS OF EXPERIMENTING

Dogs were used exclusively for the present series of tests. They were bound or held on a specially constructed holder that conformed to the body when the dog was lying on the side. They were anesthetized with nitrous oxid-oxygen mixtures through a face mask. The mask was guarded by inhalant and exhalant flutter valves. The mixture was made through a McKesson gas-oxygen apparatus, and the gases passed through a fairly long inhalant tube to the face mask. The exhaled gases were carried in a corresponding tube far enough to insure a uniform mixture of exhalant airs for sampling. Both inhalant and exhalant respiratory samples were taken for analysis. The respiratory data for a long series of preliminary tests, Experiments 1 to 30, and for the blood series, Experiments 31 to 53, reported here, are given in the preceding paper by Greene and Currey.[12]

Anesthesia was begun with a definite number of inspirations, usually ten, of pure nitrous oxid, in the attempt to remove promptly all nitrogen from the alveoli of the lungs. It was also hoped that this preliminary breathing of pure nitrous oxid would more quickly eliminate the nitrogen from its solution in the blood and body tissues. The machine was then promptly set to a mixture of gas and oxygen assumed to be efficient for anesthesia in the particular animal. Usually, from three to ten minutes were used in the preliminary stages to establish slowly an even and constant anesthesia.

Samples of respired gases and of bloods from the femoral artery and, on occasion, the vein were drawn as nearly simultaneously as possible. The actual drawing occurred as quickly after an even anesthesia was established as the vessels could be punctured. The procedure was based on the assumption that an established physical balance as between the partial pressures of the gases in the alveoli and their corresponding solutions in the blood of the pulmonary capillaries is represented in the arterial blood samples. Due to the extraordinary responsiveness of dogs to very slight variations of the percentage of oxygen mixed with the nitrous oxid, one could not always judge with fine distinctions the exact intensity of anesthesia in assumed comparative stages. In what we have called very light anesthesia, there always was some muscular movement, but uncoordinated, and some sensory reflex response. We recognize that such stages might possibly be classified as analgesias. Yet these animals, when allowed to breathe pure air, continue to exhibit unmistakable signs of unconsciousness for from ten to thirty seconds, and often much longer. As a matter of

12. Greene, C. W., and Currey, H. M.: Arch. Int. Med., this issue, p. 371.

fact, a more exact definition of the signs and degrees of anesthesia is badly needed among scientific workers in this field.

At a signal, the two gas samples and the arterial and venous blood samples were drawn, or the inhalant and exhalant airs and the arterial and venous bloods were drawn, each pair in quick succession. The venous bloods were drawn only in a small proportion of the experiments.

The blood samples were taken through Luer needles, sizes 18 to 20. Arterial punctures of the femoral are easy and sure. When venous samples were called for, it contributed to speed and accuracy if the femoral vein was first exposed by a short cut through the skin and fascias made after the onset of analgesia. The blood was drawn into a Van Slyke and Cullen sampler over sodium oxalate powder and under a layer of liquid petrolatum. A 1 by 3 inch (2.5 by 7.6 cm.) specimen tube was found to be more convenient than the longer tubes. The oxalate was dissolved in the blood by gently stirring with a rotary motion in the tube in which the sample was drawn. The analyses were made as quickly as possible, beginning immediately after the blood samples were drawn.

The bloods were analyzed in 2 c.c. samples in duplicate sets in the Van Slyke blood gas apparatus. The steps in technic are the same as described by Van Slyke and Stadie and slightly modified by Greene and Greene. By this method, the oxygen is absorbed in the apparatus and the nitrous oxid is the residual gas; hence, it is determined by difference. Two sources of error are possible by this procedure. The first depends on the extent to which the nitrogen normally in solution in the blood is thrown off during the time of anesthesia and before sampling the blood. We rely on the preliminary washing out of the lungs by pure nitrous oxid and the later substitution of pure oxygen and nitrous oxid to remove a constant stream of nitrogen from the blood and tissues, until this gas is washed out. We have assumed that this process reduces the nitrogen residue in the blood samples to within the limits of error of determination of the total residues of gas. No correction has, therefore, been made for possible residual nitrogen from this source. If the assumption should be questioned, one must reply that the maximal correction would not exceed a small and unimportant fraction of the nitrogen factor determined by Bohr, Van Slyke and the various investigators of the blood gases in more recent scientific and clinical publications. The saturation of arterial blood does not exceed a maximum of 1.3 to 1.8 per cent. by volume of nitrogen in the normal blood, but we believe it to be near 0 in our samples. We have, therefore, discarded the view of Oliver and Garrett as untenable. Hence, we have abandoned further attempt to measure the nitrous oxid directly from such small

total samples as are yielded from 2 c.c. of blood, generally between 0.4 and 0.5 c.c. or less of gases.

The far more important source of deviation arises from the great solubility of nitrous oxid in blood. The solubility in the alkaline fluids of the Van Slyke apparatus is much less than in water, or in blood alone. Yet, after gas liberation under vacuum, the nitrous oxid will rapidly be taken up in solution if, after release of the vacuum, it is allowed to stand for a few seconds before leveling and reading the gas volume. It is very necessary to trap the fluids under vacuum and to read the gas over mercury only. The untrapped fluid can, with care, be reduced to a minimum of 0.1 to 0.2 c.c. of liquid over the mercury meniscus. If the gas is read promptly, no appreciable resorption occurs.

TABLE 2.—*Nitrous Oxid and Oxygen Content of Arterial Blood and Two Samples of Venous Blood at Different Stages of Anesthesia in the Dog*

			Arterial Blood, per Cent. by Volume		Venous Blood, per Cent. by Volume		Oxygen Capacity, per Cent. by Volume	Oxygen Saturation, per Cent.	Inhalant Air			
Date	Dog No.	Experiment No.	Oxygen	Nitrous Oxid	Oxygen	Nitrous Oxid			Oxygen, per Cent.	Nitrous Oxid, per Cent.	Oxygen Pressure, Mm.	Degree of Anesthesia
3/21/23	3	31	2.4	21.7	—	—	18.9	13	6.72	93.2	51.1	Deep
3/23/23	4	32	4.8	20.6	—	—	27.5	17	5.09	94.9	38.6	Deep
3/28/23	3	33	2.1	26.6	—	—	—	11	6.74	93.2	51.2	Light
3/28/23	4	34	12.5	24.1	—	—	28	45	11.49	85.5	87.3	Light
3/30/23	4	35	17.4	19.5	—	—	—	62	6.71	93.2	50.9	Light
4/ 4/23	3	36b	12.5	26	—	—	23.2	52	3.54	96.4	26.9	Medium
4/ 6/23	4	37	18.2	19	—	—	30.9	50	10.75	85.2	81.7	Light
5/ 2/23	3	39	11.9	24.2	—	—	23	51	7.85	92.1	59.5	Light
5/ 4/23	3	40	18.8	24	—	—	27.1	70	9.04	90.9	68.7	Light
5/ 4/23	3	41	7.9	26.8	7.7	22.2	—	29	6.18	93.8	46.9	Light
5/ 4/23	3	42	8.4	24.3	—	—	23.1	36	4.4	95.6	33.4	Deep
5/14/23	3	43	15	24.5	12.9	21.9	—	65	9.6	91.4	72.9	Very light
5/ 8/23	4	44	..	20.8	—	—	24.7	—	14.6	85.4	111.6	Very light
5/ 9/23	5	46	4.7	24.5	—	—	22.3	21	3.57	96.4	27.1	Very deep

Respiratory movements have been recorded as a means of following the progress of anesthesia. The respiratory record furnishes a graphic history not only of the rate and respiratory amplitude, but also of all struggles, vocalizations and the like. As gassing becomes effective and muscular relaxation and evident anesthesia begins, the respiratory movements settle into a uniform and even rhythm and amplitude. We have come to recognize this as a most reliable index of the stage of surgical anesthesia.

The distribution of blood gases in nitrous oxid-oxygen anesthesia has been determined in twenty or more experiments. Arterial blood was used, but both arterial and venous bloods were determined in a number of experiments. The oxygen capacity also was measured and the percentage of saturation calculated. The present blood gas determinations were made in the same series of experiments used as a basis for reporting the nitrous oxid and oxygen in the respiratory airs during different degrees of anesthesia induced in dogs.[12]

An examination of the data of Table 2 reveals a surprisingly high and constant level of partial saturation of the blood with nitrous oxid during anesthesia. The amount of nitrous oxid gas varies between the extremes of 18.9 and 26.8 per cent. by volume, an average of 23.3 per cent. for Experiments 31 and 46. There is no close correspondence between the depth of anesthesia and the variation of nitrous oxid in the arterial blood within the surgical limits of very light and deep anesthesias. The very deepest anesthesias reported are Experiments 31 and 32, in which the nitrous oxid percentages by volume in the bloods are well under the average, and in Experiments 42 and 46, which have only a little over the average degree of saturation of nitrous oxid. The solubility of nitrous oxid in water, at 36 C., has been determined, by Kunerth,[13] as 44.9 per cent. by volume at 1 atmosphere of pressure. There is no evidence in the literature to indicate that whole blood will absorb any more gas than will distilled water. Apparently, nitrous oxid goes into physical solution in blood and does not form loose chemical compounds, as does oxygen. We have made preliminary determinations that indicate that whole blood takes up about 45 per cent. by volume of nitrous oxid when saturated at 25 C. The blood is therefore somewhat more than half saturated at the average of 23.3 per cent. by volume reported in the foregoing. The deviations from the average saturation are not constant, and bear little relation to the exact degree of anesthesia. In general, we have come to regard 20 per cent. by volume of nitrous oxid as the minimum present when unconsciousness is induced in the dog. We have found over 26 per cent. by volume of nitrous oxid present in only two samples of arterial blood, in Experiment 33 with 26.6 per cent., and in Experiment 41 with 26.8 per cent. by volume.

In sharp contrast with the nitrous oxid, we have found that the arterial oxygen present varies through a wide range. The table reveals arterial oxygen present all the way from 2.1 to 18.8 per cent. by volume. The first was in a relaxed animal with slower, shallow respirations which were near the point of stopping. If we were to draw analogies from the studies on anoxemia by Greene and Gilbert,[14] we should have to assume that the respiratory center was weakening from lack of oxygen. The higher oxygen volumes are all from the blood of animals in light anesthesia, indeed very light from the surgical point of view. The three dogs on which repeated experiments were made during the months of March, April and May yielded bloods of high oxygen capacity,

13. Kunerth, William: Physical Review **19**:512, 1922.

14. Greene, C. W., and Gilbert, N. C.: Responses of Circulation to Low Oxygen Tension, Arch. Int. Med. **27**:517 (May) 1921; Am. J. Physiol. **60**:155 (March) 1922.

varying between 22.3 in Dog 5 and 30.9 per cent. by volume in Dog 4. The oxygen capacity of the arterial blood also varied day by day, due to the irregular time of the experiment with reference to feed, drink, etc. Dog 3, a young and growing Airedale, apparently made a steady increase of hemoglobin from the low oxygen capacity level of 18.9 per cent. by volume, March 21, to a maximum of 27 per cent. by volume, May 4.

The absolute amount of oxygen found must be checked against the blood capacity in determining the availability of oxygen to the tissues. In fact, the capacity factor also conditions the total amount of oxygen absorbed at the lungs. It is now well known that dogs survive when breathing an air of a much lower partial pressure of oxygen than can be endured by man.[15] The higher hemoglobin content of dog's blood enables the blood to take up a correspondingly greater amount of oxygen at its lower partial pressure. The same physical and chemical factors control oxygen absorption from the nitrous oxid-oxygen mixtures. However, in the tissues, the excess of nitrous oxid may prevent oxygen utilization. The high nitrous oxid solubility in the blood and in the tissues is believed to be a factor influencing the rate of diffusion and therefore the time-quantity availability of oxygen. It is an attractive hypothesis to assume that nitrous oxid lowers the amount of oxygen fixed by hemoglobin at any particular partial pressure of oxygen in a manner comparable to that shown by Barcroft and Camis[15] for carbon dioxid. But this hypothesis has no experimental basis as yet. This question and the solubility of nitrous oxid in the tissues are both under investigation.

There are many factors that contribute to variations in the ratios of oxygen and nitrous oxid gases in the blood, and in the blood in comparison with the inhaled mixture. The chief of these are the volume per minute of the gases breathed and the volume per minute of the circulation of the blood. When the circulation is sluggish, an average stage of medium anesthesia can be attained with a gas mixture containing a higher percentage of oxygen. The low respiratory minute volume operates in the same direction. When these two fall together, the observed percentage of oxygen in the anesthetic mixture is highest of all observed. The converse is, of course, true.

As an illustration, it happens that Experiment 31 yielded the lowest nitrous oxid content, and Experiment 41 the highest nitrous oxid content of the arterial series. The first was anesthesia approaching in depth the stage in which respirations cease. The second was a good, quiet, relaxed state but far from the danger line. In these two tests on the same experimental animal, the analyzed percentage of inhaled nitrous

15. Barcroft, J., and Camis, M.: J. Physiol. **39**:118, 1909.

oxid and oxygen was essentially the same. It was noticed that this animal varied considerably in its reaction intensities during the long time of its use. It was a young animal, and both respiratory and circulatory mechanisms were unusually responsive.

In Experiments 36 and 39 on the same Airedale, the nitrous oxid-oxygen present in the blood was fairly constant, but the partial pressure of the inhalant oxygen in the latter was much higher. The reactions of the animal in the two experiments apparently varied from another angle, i. e., in the second experiment, a reduced efficiency of oxygen absorption and availability. The first experiment showed a percentage of oxygen saturation of the blood only slightly higher than the second, 53 per cent. by volume as compared with 51 per cent. by volume. The oxygen saturations are greater than in corresponding stages of simple oxygen asphyxiation. In fact, this percentage of oxygen with nitrogen of ordinary air would not produce unconsciousness in any normal animal.

TABLE 3.—*A Succession of Nitrous Oxid Experiments on a Young Airedale*

Date, 1923	Experiment No.	Inhalant Nitrous Oxid Oxygen	Exhalant Oxygen, per Cent.	Arterial		Blood Oxygen Capacity, per Cent. by Volume	Saturation, per Cent.
				Nitrous Oxid, per Cent.	Oxygen, per Cent.		
3/21	31	93.3 : 6.7	5.21	21.7	2.4	18.9	13
3/28	33	93.3 : 6.7	6.19	26.6	2.1	—	11
4/ 4	36 b	95.9 : 4.1	3.86	26	12.5	23.2	52
5/ 2	39	92.2 : 7.8	6.21	24.2	11.9	23	51
5/ 4	40	91 : 9.0	8.01	24	18.8	27.1	70
5/ 4	41	93.8 : 6.2	5.34	26.8	7.9	—	29
5/ 4	42	95.6 : 4.4	2.9	24.3	8.4	23.1	36
5/ 4	43	90.4 : 9.6	—	24.5	15	—	65

The primary deduction to be made, however, is that, in experimental procedures, considerable variation in the mixture of oxygen and nitrous oxid must be allowed to produce corresponding stages of anesthesia, if there is a corresponding variation in efficiency of the two great mechanisms of respiration and circulation. This relation is reciprocal.

There also must be adjustment as between the percentage of oxygen in nitrous oxid mixtures made from time to time when an animal continues in surgical anesthesia. This, too, depends not so much on metabolism per se as on the deviations in efficiency of the circulation and of the respiration, because of the duration of the anesthesia. For example, in deep, prolonged anesthesia the respiratory volume and rate both progressively decrease. If no other factor operated, this would throw the animal into a still deeper stage of anesthesia, and would, in the end, endanger respiratory rhythm itself. Similar conditions apparently hold with reference to the circulation, particularly when the amount of oxygen is on the borderline of inadequacy. Under these circumstances, the ordinary cycle of events which occurs in extreme

anoxemia is initiated in nitrous oxid anesthesia. The animal may go to the wall rapidly unless the percentage of oxygen is promptly increased and the animal adequately reoxygenated. In these crises, it is not the percentage of variation of nitrous oxid in the mixture but the availability of oxygen at the moment in the tissues which determines the outcome of the crisis.

In Table 3, we have assembled eight experiments on one young Airedale extending over a period of seven weeks. The degree of anesthesia varied from very light with muscular movements present to very deep surgical anesthesia. This table emphasizes the points we would stress. In the first place, the concentration of nitrous oxid found in the blood is surprisingly uniform. It does not vary directly with the stage of surgical anesthesia, in such slight variations as do occur. Comparing Experiments 41 and 42, the first, with the greater concentration of nitrous oxid, had the less profound anesthesia.

These experiments sharply contrast the relation of oxygen percentages to the stage of anesthesia. With an adequate concentration of nitrous oxid in the blood to induce anesthesia, the stage or depth of anesthesia varies directly with the amount of oxygen present. It follows that the control of anesthesia in all gas anesthesias is by the control of oxygen. The problem of anoxemia is always a factor. However, the amount of oxygen present in arterial blood in the lighter and surgical phases is somewhat higher in nitrous oxid-oxygen anesthesia than during oxygen want induced by diminishing the oxygen partial pressures in atmospheric air to a level that will not sustain consciousness.

THE BLOOD PHOSPHORUS IN CHRONIC MYELOGENOUS LEUKEMIA, ESPECIALLY AS INFLUENCED BY ROENTGEN-RAY THERAPY *

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An abnormal phosphorus metabolism in leukemia has been recognized for over a quarter of a century, and yet very few observations are recorded in the literature relative to the phosphorus content of the blood in this type of disease. Moraczewski [1] in 1898 called leukemia, rather loosely, a "phosphorus and nitrogen disease" and reported not only an increased urinary excretion of phosphorus and nitrogen, but also an abnormally high phosphorus content of the blood. Though numerous studies since then, such as those of Musser and Edsall [2] and Knudson and Erdos,[3] have clearly demonstrated the excessive output of phosphorus in the urine, there seems to be no report of any systematic investigations of the blood phosphorus in chronic myelogenous leukemia entailing an intensive study of cases over a considerable period of time. This is presumably due to the fact that it is but recently that relatively simple methods have become available for determining the blood phosphorus, while those for determining urinary phosphorus have been satisfactory for some years.

The early investigators of the problem failed to distinguish the types of leukemia and consequently not even their few data can be evaluated satisfactorily. Moreover, inasmuch as nearly all of the reported observations regarding blood phosphorus in myelogenous leukemia have had to do either with the whole blood alone or with the plasma alone, it is still an open question whether there exists in this disease, such as exists in rickets, a true disturbance of the phosphorus metabolism, or whether the high level of blood phosphorus is incidental to the leukocytosis.

The present study was undertaken in an attempt to determine whether the phosphorus content of the blood is merely a function of the leukocytosis, or whether it may not be looked on as a manifestation of some more

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* This paper is No. 35 of a series of studies in metabolism from the Harvard Medical School and allied hospitals. The expenses of this investigation have been defrayed (in part) by a grant from the Proctor Fund of the Harvard Medical School for the study of chronic diseases.

1. Moraczewski, W.: Arch. f. path. Anat. **159**:221, 1898.
2. Musser, J. H., and Edsall, D. L.: Univ. Penn. M. Bull. **18**:174, 1905.
3. Knudson, A., and Erdos, T.: Boston M. & S. J. **176**:503 (April 5) 1917.

nearly fundamental phase of the activity of the disease. Bearing in mind also the beneficial influence of radium and roentgen-ray irradiation in myelogenous leukemia, it was hoped that by an investigation of the blood phosphorus changes following such irradiation some light might be thrown on the mechanism by which this type of therapy acts. Accordingly, a study of the total and inorganic phosphorus contents of the whole blood, plasma and corpuscles was made in a series of nineteen cases of chronic myelogenous leukemia in various stages of the disease, before, during and after short wave length roentgen-ray treatment. For the purpose of comparing the influence of irradiation therapy on the blood phosphorus in myelogenous leukemia with its influence on the blood phosphorus in cases showing no important degree of leukocytosis, five cases of carcinoma were studied also. Three had carcinoma of the breast, one of the tongue and one of the stomach.

The means of the phosphorus values of the blood of normal persons, reported in a previous paper,[4] are submitted here and are in accord with the findings of other workers.

METHODS

Blood from a vein was withdrawn under paraffin oil, 0.1 c.c. of a 20 per cent. solution of neutral potassium oxalate for each 15 c.c. of blood being used as an anticoagulant. After thorough mixing, samples of the whole blood were withdrawn from the container for the determinations of total and inorganic phosphorus and for the estimation of the percentage by volume of corpuscles. The remainder of the blood was centrifugalized for twenty minutes at eighteen hundred revolutions per minute, when the supernatant plasma was removed for analysis of its content of total and of inorganic phosphorus.

The percentage volume of corpuscles was estimated by centrifugalizing for thirty minutes at two thousand revolutions per minute, about 1 c.c. of whole blood in a carefully calibrated, graduated tube about 5 mm. in diameter, and then reading the fraction of the total volume of blood made up by the corpuscles. Also, in each case an approximate estimation was made of the fraction of the total volume of corpuscles made up by the white cells and blood platelets.

The total phosphorus in the whole blood and plasma was determined by the method of Bloor[5] and the inorganic phosphorus by the method of Bell and Doisy.[6] Having determined the total and inorganic phosphorus contents of the whole blood and plasma and the percentage

4. Buckman, T. E.; Minot, G. R.; Daland, Geneva; and Weld, M.: Blood Phosphorus: Its Relation to Cancer and Anemia, Arch. Int. Med. **34**:181 (Aug.) 1924.

5. Bloor, W. R.: J. Biol. Chem. **36**:33 (Oct.) 1918.

6. Bell, R. D., and Doisy, E. A.: J. Biol. Chem. **44**:55 (Oct.) 1920.

volume of cells, the phorphorus contents of the cells were calculated from these data. The phosphorus determinations are recorded as milligrams of orthophosphoric acid, H_3PO_4, per hundred cubic centimeters of whole blood, plasma or cells.

The hemoglobin percentage was determined by means of a standardized Sahli instrument and the values given are expressed in terms of the Haldane scale (18.5 per cent. by volume of oxygen combining capacity per hundred per cent. hemoglobin). Enumeration of the numbers of corpuscles per cubic millimeters of blood and the differen-

TABLE 1.—*Blood Phosphorus in Normal Persons and in Nonirradiated or Not Recently Irradiated Cases of Chronic Myelogenous Leukemia*

Case	Red Blood Cells in Millions	Hemoglobin, per Cent.	White Blood Cells in Thousands	Per Cent. Immature White Blood Cells	Total Phosphoric Acid in 100 C.c.			Inorganic Phosphoric Acid in 100 C.c.		
					Whole Blood	Plasma	Cells	Whole Blood	Plasma	Cells
Mean value five healthy adults	4.8	96	8.2	..	119.3	29.6	233.2	11.0	10.4	12.3
1*	3.5	85	14.6	2	136.6	44.9	342.0	13.5	14.5	11.3
2*	6.4	98	29.9	10	101.9	21.6	194.4	24.3	16.0	34.0
3*	3.9	94	50.0	21	80.2	24.2	191.5	9.8	8.1	12.5
4	4.4	90	78.6	6	.....	....	.....	14.3	....	....
5*	2.6	47	103.0	14	60.2	34.2	139.0	13.2	11.7	17.7
6*	4.7	75	119.2	28	146.5	18.4	316.0	11.1	11.4	10.8
7*	3.6	84	134.4	41	212.5	27.1	506.1	20.1	12.9	31.6
8*	4.5	65	181.6	49	121.0	43.4	254.0	29.6	16.6	52.2
9*	3.4	59	190.0	30	.....	....	.....	14.7	11.2	22.1
10	3.9	74	192.0	32	129.9	26.0	348.5	22.3	14.0	39.6
11	2.4	56	216.0	41	.....	....	.....	14.9	11.6	19.6
12	3.0	54	230.0	18	.....	....	.....	13.6	9.1	22.2
13	4.1	65	270.0	28	156.7	22.0	318.0	31.5	16.8	47.2
14	3.3	58	281.0	55	236.4	32.7	514.4	31.6	11.5	59.3
15	3.0	47	312.0	46	.....	....	.....	9.5	7.2	12.6
16	3.5	68	326.0	56	128.2	20.3	283.0	23.1	12.0	39.0
17	3.3	60	353.0	52	245.2	39.8	463.0	30.2	14.5	46.8
18	2.6	50	406.0	75	365.9	20.5	852.0	18.5	3.6	39.4
19	3.3	60	434.0	42	247.5	63.0	460.0	19.1	10.3	32.8
Mean value	3.6	68	206.3	..	169.8	31.2	370.4	19.2	11.8	30.5

* Treated by irradiation, but at a time sufficiently remote from the present observations (i. e., two months or more) to preclude the influence of such treatment, per se, on the phosphorus metabolism.

tial counts of at least 200 white corpuscles in each instance were made in the usual manner. All myeloid white cells less mature than metamyelocytes were classified together as "immature white cells."

OBSERVATIONS

1. *The Findings in Nonirradiated and Not Recently Irradiated Cases of Chronic Myelogenous Leukemia.*—In Table 1 are recorded the results obtained in nineteen cases of chronic myelogenous leukemia. Those designated by an asterisk had received irradiation treatment, but at a time sufficiently remote (over two months) from these observations to preclude any influence of irradiation, per se, on the phosphorus metabolism. The other cases had received no irradiation treatment.

It is evident from inspection of this table that although the levels of total and inorganic phosphorus in the whole blood in the cases of leukemia are generally very much elevated above the mean normal values, the plasma shows no such increase in its content of phosphorus. The mean values for total and inorganic phosphorus in the whole blood of the cases of leukemia are 169.8 mg. and 19.2 mg. per hundred cubic centimeters of blood, respectively, as contrasted with the mean normal values of 119.3 mg. and 11.0 mg. On the other hand, the mean values for total and inorganic phosphorus of the plasma from the leukemia cases are 31.2 mg. and 11.8 mg. per hundred cubic centimeters of plasma, respectively, as contrasted with mean normal values of 29.6 mg. and 10.4 mg. In other words, the increased phosphorus content of the whole blood in chronic myelogenous leukemia is due almost entirely to the increased phosphorus content of the corpuscles.

It is true that in cases of leukemia greater variations occur than in normal persons in the plasma contents of both the total and inorganic phosphorus, and that occasionally very high values are observed: 163 mg. of total phosphorus per hundred cubic centimeters of plasma in Case 19, and 16.8 mg. of inorganic phosphorus per hundred cubic centimeters of plasma in Case 13. These variations in the total and inorganic phosphorus contents of the plasma apparently bear no relation to one another, nor to the phosphorus content of the corpuscles, nor to any of the factors discussed later which are associated with the increase of the corpuscular phosphorus. It could be supposed that the excessive demand for phosphorus caused by the rapid cell proliferation might lead to a paucity of phosphorus in the plasma as suggested by Case 18, in which the total phosphorus content of the blood rose to the very high value of 366 mg. per hundred cubic centimeters of whole blood and the total phosphorus content of the plasma dropped to 20.5 mg. per hundred cubic centimeters. But this conception is not borne out by the findings in the other cases. On the contrary, the idea might be entertained that enrichment of the plasma in phosphorus should occur as a result of the abnormally rapid disintegration of the cells in the peripheral blood or, teleologically, from the increased demand for phosphorus on the part of the bone marrow. Yet the figures attest that neither of these conceptions is tenable.

The phosphorus metabolism in myelogenous leukemia does not seem to be implicated in the sense that it is in such disorders as rickets, fracture, and certain types of arthritis. If it is, the transport of phosphorus must be effected by the corpuscles themselves, or else in leukemia the equilibrium is maintained carefully between the withdrawal of phosphorus from the plasma by the blood forming tissue, and, the return thereto from disintegrated cells and the subsequent elimination therefrom by the kidneys. Under the conditions of the

observations reported, the plasma phosphorus content in nonirradiated or not recently irradiated cases of chronic myelogenous leukemia is independent of the phosphorus contained in the corpuscles.

More interesting variations are found in the values for corpuscular phosphorus. The phosphorus content of the corpuscles might be and apparently is influenced by several factors among which are: (1) the volume of blood platelets; (2) the volume of adult and of immature red blood corpuscles of different types and the percentage of hemoglobin, and (3) the volume of the mature and of the different forms of immature white cells.

It has been shown[7] that the platelets contain a relatively large amount of phosphorus. As the number of these elements is often increased and sometimes markedly so in chronic myelogenous leukemia, it is reasonable to suppose that when such is the case the elevation of the phosphorus content of the whole mass of cells may be due, in part, to an increase in the volume of these corpuscles. However, it is impossible to determine to what extent this factor is operative for no suitable method exists for the quantitative separation of the total mass of platelets from the other corpuscles. Again, and for the same reason, the fraction of the total phosphorus content of all the cells contributed by the red blood corpuscles can be apprehended only approximately. There is, moreover, another difficulty and that is the presence of large numbers of immature red blood cells in many cases of myelogenous leukemia and these undoubtedly contain more phosphorus than do adult red cells. Thus we have observed the total phosphorus at least as high as 374 mg. per hundred cubic centimeters of red cells in a case of myelogenous leukemia with many immature red corpuscles, and a value for total phosphorus as high as 560 mg. per hundred cubic centimeters of red cells in a case of chronic hemolytic jaundice showing 30 per cent. of the red cells to be reticulocytes.[4] Commensurately high values also have been observed by us in cases of anemia due to blood loss, in which there was an increase in the number of young red cells.

Finally, the same difficulties which are met with in considering the phosphorus content of the platelets or of the red corpuscles recur in considering that of the white cells. The volume of total white cells made up by immature white cells is indeterminate, and even though quantitative separation of the leukocyte fraction of the corpuscles were possible, the methods available for the analysis of such a mass of leukocytes for its content of phosphorus are not satisfactory.

Nevertheless, from a purely statistical consideration of the data, there is an apparently definite relationship between the total phosphorus

7. Mathews, A. P.: Physiological Chemistry, Ed. 3, New York, William Wood & Co., 1920, p. 467. Hammarsten, O.: Physiological Chemistry, Trans. by Mandel, Ed. 6, New York, John Wiley & Sons, 1911, p. 295.

content of the entire cell volume and the percentage of what have been termed immature white cells. The greater the number of immature white cells, the greater becomes the total phosphorus content of the whole mass of corpuscles. This relationship is illustrated in Chart 1 which shows, also, a similar but less striking relationship between the inorganic phosphorus content of the corpuscles and the percentage of immature leukocytes. The data also show that an analogous but distinctly less definite relationship obtains between the total phosphorus content of the whole corpuscles and the total white cell count or the volume of the white cell fraction of the corpuscles, both of which are very roughly proportional to the percentage of immature white cells.[8]

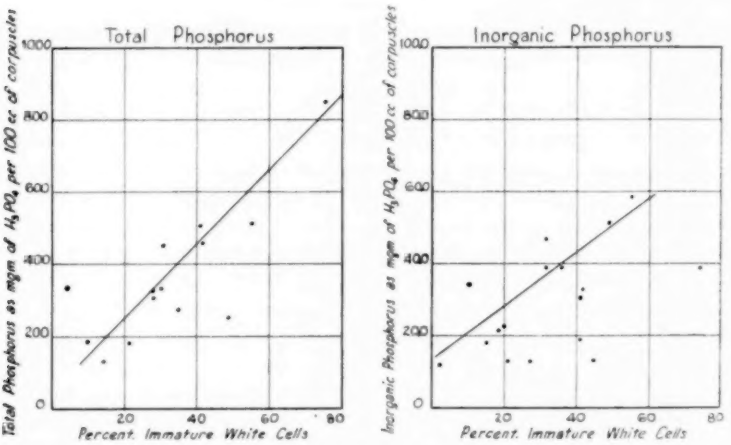

Chart 1.—The relationship between the phosphorus content of the corpuscles and the percentage of immature white cells.

The relationship between the immaturity of the white cells and the phosphorus content of the corpuscles is a similar one to that which obtains between the basal metabolic rate of the patient and the number of immature bone marrow cells, especially the leukocytes in the peripheral blood, as pointed out by Gunderson [9] and substantiated by further observations at this hospital. These two relationships are represented together in Chart 2. Although the percentage increment in the basal metabolic rate of the patient and the percentage increment in the

8. It is not intended to imply here that any close parallelism exists between the total number of white corpuscles per cubic millimeter of blood and the percentage of immature white corpuscles. The association is only a very general one, and discrepancies are too numerous and too marked to permit the use of the count of the white cells in any given case as an index of the degree of maturity of these cells.

9. Gunderson, A. H.: Boston M. & S. J. **185**:785 (Dec. 29) 1921.

total phosphorus content of the whole corpuscles with increasing numbers of immature white cells follow somewhat different graphs, it is probable that both are different manifestations of the same phenomenon; namely, the presence in the blood of increased numbers of young cells. Of the two, the phosphorus content of the corpuscles is the more sensitive index of the degree of immaturity of the blood, inasmuch as the basal metabolic rate of the patient is undoubtedly influenced by compensatory factors operating to depress it.

2. *The Early Effects of Short Wave-Length Roentgen Therapy on the Blood Phosphorus.*[10]—Observations on the blood phosphorus contents of

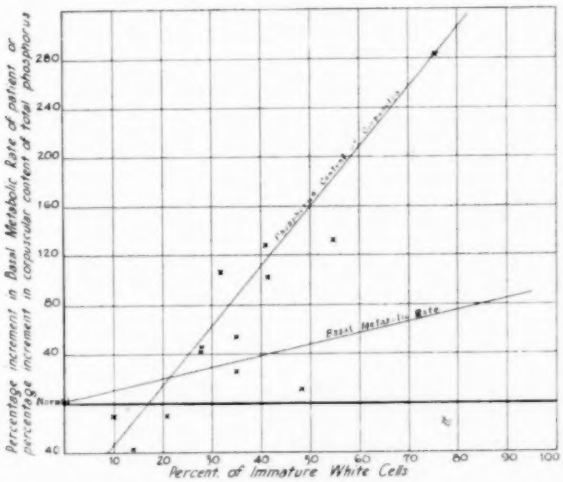

Chart 2.—The relation of the percentage increment in the total phosphorus content of the corpuscles and the percentage increment of the basal metabolic rate of the patient to the percentage of immature white corpuscles. The graph of the phosphorus content of the cells is the median obtained from the results shown in Table 1. The graph of the basal metabolic rate is the median obtained from Gunderson's data. The crosses represent values for corpuscular content of total phosphorus.

the patients with chronic myelogenous leukemia and cancer were made not only before irradiation and at periods remote from the time of therapy, as stated in the foregoing, but also at intervals varying from a few hours to many days following the application of the roentgen rays. Concerning the effects of irradiation that occur within a few hours

10. In each instance the high voltage, constant potential, short wave length roentgen ray described by Duane (Am. J. Roentgenol. **9**:781, 1922) was used. As a rule, from three to four exposures on successive days were given. The average dose of each exposure was about 600 electrostatic unit seconds, exclusive of secondary radiation.

TABLE 2.—*The Phosphorus Content of Blood in Myelogenous Leukemia and Cancer Before, During and Following Short Wave Length Irradiation Therapy* *

Case	Diagnosis	Date		Red Blood Cells in Millions	Hemoglobin, per Cent.	White Blood Cells in Thousands	Per Cent. Immature White Blood Cells	Total Phosphoric Acid in 100 C.c.			Inorganic Phosphoric Acid in 100 C.c.		
								Whole Blood	Plasma	Cells	Whole Blood	Plasma	Cells
13	Chronic myelogenous leukemia.....	9/20	Roentgen-ray treatment.....	4.1	65	270	28	155.7	22.7	318.9	31.5	16.8	47.2
		9/21	Roentgen-ray treatment.....	...	...	270	..	224.1	37.2	472.0	29.0	17.6	41.8
		9/22	.....	...	...	176	11	149.0	43.6	306.0	22.9	19.4	27.5
		9/24	Roentgen-ray treatment.....	4.9	91	54	6	113.1	52.4	187.3	25.5	24.6	24.3
		9/25	Roentgen-ray treatment.....	...	...	...	..	.....	....	.....	....	....	....
		9/27	.....	5.4	110	29	6	134.2	57.7	231.0	22.1	17.1	24.0
		10/ 4	.....	3.9	...	10	14	75.0	24.6	165.0	10.3	7.9	14.7
17	Chronic myelogenous leukemia.....	2/28	Roentgen-ray treatment.....	3.3	60	353	32	245.2	39.8	463.0	30.1	14.5	46.8
		2/29	Roentgen-ray treatment.....	3.7	65	304	30	326.1	27.4	738.0	29.7	14.9	49.8
		3/ 1	.....	3.6	...	252	..	135.2	30.5	270.5	22.4	14.6	33.1
		3/ 2	.....	3.4	75	160	20	.....	....	.....	....	....	....
		3/ 3	.....	...	69	133	7	186.0	25.3	472.5	38.6	15.7	27.6
		3/14	.....	4.3	82	18	3	102.6	46.5	209.2	12.4	12.4	12.2
14	Chronic myelogenous leukemia.....	3/19	Roentgen-ray treatment.....	3.3	58	281	55	236.4	32.7	514.4	31.6	11.5	59.3
		3/20	Roentgen-ray treatment.....	3.0	68	168	34	127.1	39.7	327.7	22.5	14.8	39.2
		3/21	Roentgen-ray treatment.....	...	...	...	..	.....	....	.....	....	....	....
		3/23	.....	...	65	99	11	.....	....	.....	....	....	....
		3/24	.....	3.4	79	47	3	.....	....	.....	....	....	....
		3/26	.....	2.9	82	22	7	96.5	33.3	239.4	10.0	8.2	14.3
		3/28	.....	3.4	81	7	5	.....	....	.....	....	....	....
6	Chronic myelogenous leukemia.....	10/15	Roentgen-ray treatment.....	4.7	75	119	28	140.0	18.5	316.0	11.1	11.4	10.8
		10/16	Roentgen-ray treatment.....	4.9	84	112	12	157.0	22.3	319.0	18.2	11.4	26.3
		10/17	Roentgen-ray treatment.....	4.7	72	81	18	137.0	26.9	264.0	16.2	12.3	20.9
		10/24	.....	4.3	88	13	7	116.0	16.0	270.0	10.3	7.1	15.2

16	Chronic myelogenous leukemia	8/27	Roentgen-ray treatment	3.5	...	325	32.5	120.0	20.0	286.0	23.0	12.0	39.0
		8/29	Roentgen-ray treatment	2.9	70	210	20	225.0	46.5	500.0	23.0	13.5	36.0
		9/ 7		4.2	82	15	20	95.0	30.5	250.0	9.0	8.0	10.0
3	Chronic myelogenous leukemia	12/ 3	Roentgen-ray treatment	3.9	90	50	20	90.0	24.0	190.0	10.0	8.0	12.5
		12/ 4	Roentgen-ray treatment	...	100	45	..	220.0	68.5	436.0	10.5	12.5	8.5
		12/ 5	Roentgen-ray treatment	5.1	103	30	12	165.0	22.0	360.0	13.0	12.0	14.0
		12/ 6		...	90	25	17.5	120.0	32.0	225.0	12.5	13.0	12.0
		12/11		4.5	102	30	12.5	110.0	21.5	235.0	9.0	7.0	12.0
20	Cancer of the breast	11/ 4		4.2	97	6.3	..	.....	....	.....	....	....	....
		11/ 5	Roentgen-ray treatment	...	...	...	..	110.0	29.0	210.0	11.5	12.0	10.0
		11/ 6	Roentgen-ray treatment	4.4	97	5.1	..	90.0	62.0	145.0	12.0	11.5	13.2
		11/ 7		4.6	97	6.4	..	120.0	42.0	235.0	12.5	12.0	14.1
21	Cancer of the breast	11/12	Roentgen-ray treatment	4.9	98	11.1	..	85.0	42.0	155.0	13.0	11.5	16.5
		11/13	Roentgen-ray treatment	4.9	99	12.2	..	95.0	62.0	140.0	14.5	12.5	18.0
		11/14		4.6	97	6.8	..	105.0	35.0	215.0	11.5	9.5	15.0
		11/16		5.0	104	18.3	..	115.0	49.0	210.0	14.0	13.0	16.0
22	Cancer of the breast	10/22	Roentgen-ray treatment	6.1	103	8.2	..	125.0	48.0	245.0	9.0	11.0	6.0
		10/23	Roentgen-ray treatment	5.4	110	9.2	..	175.0	78.0	340.0	13.0	13.0	13.0
		10/24	Roentgen-ray treatment	4.7	104	8.5	..	90.0	71.0	125.0	10.0	10.0	9.0
		10/26		4.4	106	8.1	..	85.0	48.0	140.0	11.0	11.0	12.0
		11/ 8		5.0	110	9.4	..	115.0	42.0	260.0	10.5	10.5	10.5
23	Cancer of the stomach	10/26	Roentgen-ray treatment	4.3	68	20	..	150.0	26.0	420.0	9.0	11.0	8.0
		10/27	Roentgen-ray treatment	4.5	72	21.6	..	85.0	50.0	160.0	13.0	11.0	19.0
		10/29		3.9	68	17.8	..	70.0	22.0	190.0	12.5	11.5	14.0
		11/15		5.2	77	15	..	70.0	32.0	155.0	....	....	....
24	Cancer of the tongue	2/21		3.8	78	10.7	..	55.0	28.5	105.0	12.0	9.0	18.0
		2/23	Roentgen-ray treatment	...	...	...	..	.....	....	.....	....	....	....
		2/25	Roentgen-ray treatment	4.6	88	13.8	..	90.0	43.0	170.0	13.0	13.0	12.0
		2/28		...	88	7.2	..	100.0	46.0	220.0	14.0	14.0	14.0

* In each case, the determinations on the blood were made prior to the roentgen-ray treatment; in many of the cases, this was done on the same day that the treatment was given.

following exposure, the data accumulated show too many and too extreme variations to warrant any conclusions at present. These data are not reported. The course of events following the initial disturbances due to irradiation is represented, however, in Table 2, in which are shown the findings in the five cases of cancer and in six typical cases of chronic myelogenous leukemia. Even these observations together with those on the other thirteen cases of leukemia do not permit of an entirely satisfactory recapitulation. Nevertheless, certain definite tendencies both in the cases of cancer and in the cases of leukemia are evident.

Within twenty-four hours following the first exposure to irradiation in a given series of treatments there occurs, generally, an initial rise in the level of the total phosphorus content of the plasma and, frequently, a rise in the total phosphorus content of the corpuscles. These initial elevations may be followed within a day or two by sharp declines to normal or even subnormal levels of total phosphorus values for both plasma and cells.

Following subsequent exposures to irradiation in the same series of treatments, the total phosphorus content of the plasma usually rises again above the upper normal limit (about 35 mg. per hundred cubic centimeters) or, should it not have fallen following the first exposure, it rises to still higher levels provided that the value for the total phosphorus content of the plasma was below the upper normal limit at the time the first treatment was given. Under such circumstances the total phosphorus content of the cells continues to fall. But if the value for the plasma content of total phosphorus, especially at the time of subsequent treatments, is above the upper normal limit, then it is generally true that following subsequent treatments the total phosphorus content of the plasma remains stationary or falls; whereas the total phosphorus content of the cells rises and this rise in the cell phosphorus is maintained until such a time as the plasma phosphorus has declined to values within normal limits.

The inorganic phosphorus contents of the plasma and cells, though subject to marked variations, show a distinct tendency after irradiation to approach normal or subnormal values as the abnormal and excessive numbers of formed blood elements.

The alterations after irradiation in the total and inorganic phosphorus of the whole blood and cells in leukemia are much more striking than those observed in cancer. However, the changes that occurred in the cases of cancer, though relatively slight, were similar to those in the cases of leukemia and serve but to emphasize the before mentioned generalizations regarding blood phosphorus following irradiation. In a patient with cancer, irradiation at first causes a decrease in the leukocyte count and a decrease of immature blood cells, as it usually does to a

vastly greater degree in leukemia. It is to be noted that both in leukemia and cancer the corpuscular phosphorus may actually rise, in the former, markedly, in the latter, very slightly after irradiation in the face of a falling white cell count and a falling percentage of immature bone marrow cells.

Although it is impossible in the present state of our knowledge to explain the fluctuations in the values for inorganic phosphorus in the blood following irradiation in cancer and leukemia, it is suggested that the following explanation might account for the observed changes in the total phosphorus contents of the corpuscles and plasma. The destruction of cells incident to irradiation and certainly not confined to the blood cells results in the liberation and excretion of large amounts of phosphorus. This phosphorus may be largely or wholly taken up by the plasma, provided the conditions for elimination are favorable, as evinced by the finding of a normal plasma content of phosphorus before treatment. But if cell destruction has been excessive, or if, as shown by an abnormally high phosphorus content of the plasma before treatment, conditions for elimination are unfavorable, then the liberated phosphorus may be stored temporarily in the corpuscles and particularly in the red blood corpuscles, until the excess of phosphorus in the plasma has been eliminated. This conception is in part borne out by the work of Ordway, Tait and Knudson [11] who found the increase in the phosphorus content of the urine after irradiation to be sustained, particularly in myelogenous leukemia, even after the blood histology had become approximately normal.

MORE REMOTE EFFECTS OF IRRADIATION

It has been shown [4] that barring those conditions in which the phosphorus metabolism is truly disturbed, the total phosphorus content of the whole blood is a function of its hemoglobin content. As in other conditions, so it is in chronic myelogenous leukemia when, through irradiation therapy, or otherwise, the white corpuscles have been reduced to normal and the early effects of irradiation have subsided. This trend of events is illustrated by the cases represented in Chart 3. In this chart the heavy line indicates the ideal values for the total phosphorus content of the whole blood for different levels of hemoglobin, as previously determined by us.[4] The numbered lines represent the actual course of the total phosphorus following irradiation, in each of four of the leukemia cases and in two of the cases of cancer.

The values for the total phosphorus content of the whole blood (ordinates) are plotted against the values for the hemoglobin content of the blood (abscissas). The chart shows that both in the cases of

11. Ordway, T.; Tait, Jean; and Knudson, Arthur: Albany M. Ann. **41**:1 (Jan.) 1920.

cancer and leukemia the actual values for total phosphorus in the whole blood approach the idea values, in the former before irradiation and again as soon as the disturbances incident to irradiation have subsided; in the latter as soon as the total number of white corpuscles and the immature white corpuscles have been reduced to essentially normal levels.

In leukemia, this reduction of the number and immaturity of the white cells is evident very shortly following exposure to irradiation, and is usually complete in from five days to a few weeks thereafter. Thus the blood remains during remission. Finally, as relapse occurs, as illus-

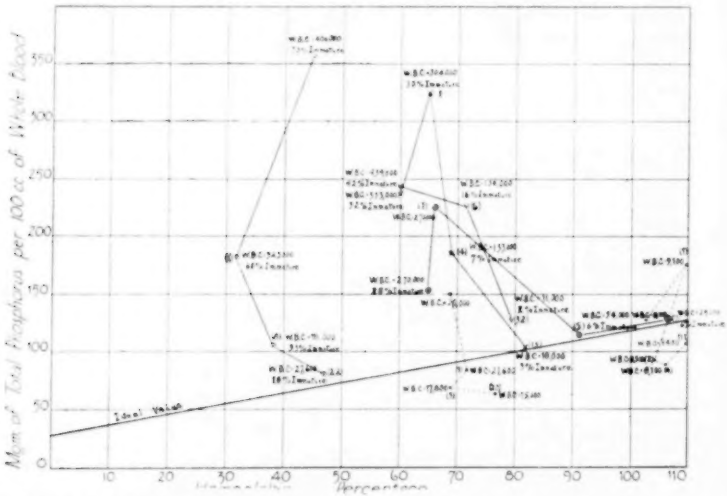

Chart 3.—The relationship between the total phosphorus content of the whole blood and the percentage hemoglobin content of the blood in two typical cases of cancer (dotted lines) and four typical cases of chronic myelogenous leukemia (solid lines). The small numbers refer to number of days elapsed since first exposure to irradiation therapy. No number is appended to observations made before exposure to irradiation.

trated by Cases 1 to 9 inclusive, the blood phosphorus values increase and again become determined largely by the same factors (immaturity of cells, volume of cells, etc.) which had controlled them before exposure of the patient to irradiation.

SUMMARY

1. A study of the blood phosphorus was made in nineteen cases of chronic myelogenous leukemia and in five cases of cancer before, during and following roentgen-ray therapy.

2. In nonirradiated or not recently irradiated cases of chronic myelogenous leukemia, the total phosphorus content of the whole blood is

markedly elevated. This increase is due almost entirely to the increased total phosphorus content of the corpuscles. The mean value for the total phosphorus content of the plasma, in spite of wide individual variations, was found to be within normal limits.

3. The total phosphorus content of the corpuscles in chronic myelogenous leukemia is dependent in part on the number of white corpuscles present, but particularly on the number of immature myeloid cells, especially white corpuscles.

4. Relationships are illustrated between the numbers of immature cells in the blood and the basal metabolic rate of the patient, and between them and the corpuscular content of total phosphorus. The total phosphorus content of the corpuscles is a more sensitive index of their immaturity than the patient's basal metabolic rate.

5. Changes in the blood phosphorus occur as a result of roentgen-ray therapy given to a patient with leukemia or cancer. As a result of tissue destruction incident to such treatment, a transient, but often, in leukemia, a marked increase of the total phosphorus content of both plasma and cells generally occurs, the latter apparently serving as a temporary reservoir for the excess of phosphorus, which for the moment cannot be taken care of by the plasma.

6. In chronic myelogenous leukemia, when the initial disturbances following irradiation have subsided, the corpuscular content of total phosphorus falls to normal levels as the numbers of immature cells in the blood stream diminish. Thereafter, as long as the numbers of white cells and immature cells remain within essentially normal limits, the total phosphorus content of the whole blood is in proportion to the percentage hemoglobin content, as is the case in other conditions.

7. No significant variations were noted in the inorganic phosphorus contents of plasma or corpuscles before, during, or after roentgen-ray therapy in leukemia or cancer.

THE PROGNOSIS OF CHRONIC INFECTIOUS ENDOCARDITIS *

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According to reports in the literature, chronic infectious endocarditis is usually fatal. Billings,[1] observed only one recovery in fourteen patients, and all of the ten reported by Osler [2] died. Of the 150 patients with subacute bacterial endocarditis reported by Libman,[3] 146 died, and recently Thayer [4] reported 206 cases terminating fatally. Contrasted with these are other statistics mentioning a lower death rate. Four patients treated with sodium cacodylate by Capps [5] are reported well after three years. Graham, Ollie and Detweiler [6] have observed twenty-three patients for a period of nine years, and of these at least twenty are living. The presence of bacteria in blood cultures of patients with endocarditis is generally regarded as indicating a grave prognosis. This idea is not altogether in harmony with results obtained at St. Luke's Hospital, Chicago. Of fifty-seven patients with bacterial endocarditis twenty-four are living and many of them seem to have recovered from the heart valve infection. The other thirty-three are dead.

CLINICAL DIAGNOSIS

Any discussion of the results of bacterial endocarditis obviously hinges on the proper clinical diagnosis of the disease. A progressive valvular lesion, fever, and the clinical signs of embolism of the brain, spleen, or kidneys are important symptoms, but in order to make the diagnosis certain, these clinical manifestations need to be supported by recovering the specific bacteria in cultures from the blood of such patients. In fact, demonstrating bacteria in the blood by cultures is probably the most conclusive evidence of an infectious endocarditis.

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* From the Medical Service and Pathological Laboratory of St. Luke's Hospital, Chicago.

1. Billings: Arch. Int. Med. **4**:409, 1909.
2. Osler: Quart. J. Med. **2**:219, 1909.
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In the number of patients herein reported with chronic infectious endocarditis studied at St. Luke's Hospital, pathogenic bacteria were recovered by cultures of the blood of each one. Each one had a heart murmur. All had fever not explained by extracardial lesions, and many had an anemia and the clinical signs of embolism.

Almost all of the twenty-four patients living are without fever and other signs of infection, and many of them are able to do light work. One has been well for thirteen years, two for seven years, and one for two and a half years. Thirteen are in the second year and seven are in the first year of clinical observation.

The clinical progress of the patient who died differed markedly from that of those living. This difference has made possible a prediction regarding the outcome soon after the disease is recognized. Those who died had a higher fever and more marked symptoms of sepsis. Twenty-six of the thirty-three had a prolonged irregular septic type of fever, the temperature ranging from 101 to 105 F. Twelve of these thirty-three had chills. Of the twenty-four living, none had fever exceeding 101 F., the maximum being from 99 to 100 F., except during an intercurrent infection. The clinical record of none of these mentions chills.

Anemia and symptoms of embolism occur in the patients of each group. But the anemia is usually more pronounced, and symptoms of embolism are more frequent in those of the septic type. The urine of twenty of the thirty-three who died contained red blood cells; twelve had petechiae of the skin; twelve a palpably enlarged spleen; eleven a severe anemia (red blood cells 3,500,000 or less, hemoglobin 65 per cent. or less); five painful cutaneous nodes; three retinal hemorrhages; two hemiplegia; and two club fingers. Of the twenty-four living, in only eight were there red blood cells in the urine; three had petechiae of the skin; four a palpably enlarged spleen; seven a marked anemia; none had painful cutaneous nodes or retinal hemorrhages; three had hemiplegia and none club fingers.

Although blood was often found in the urine of patients with the milder type, the red corpuscles in the urine of these patients were present in smaller numbers and only after repeated examinations. In contrast to this, blood was found more regularly and in larger quantities in the urine of the patients who died.

MODE OF ONSET

The mode of onset is noteworthy. In the fatal group there is a rapid development of weakness and anemia, with fever of the septic type and often with signs of cardiac decompensation. A chill or symptoms of an embolus, in a few instances, is the first manifestation of the

disease. The onset of the disease in the twenty-four living patients was in no instance marked by a chill and was accompanied, as before mentioned, by a lower fever.

Acute polyarticular rheumatism was associated with the endocarditis in ten of the patients with the milder type. Whether the arthritis preceded or followed the endocarditis was usually difficult to determine. The endocarditis seemed to be the antecedent in one patient. From a clinical standpoint the disease in many of this group cannot be distinguished from a rheumatic endocarditis. The onset of the disease of fourteen patients with the milder type was not associated with arthritis. Only four of the thirty-three patients who died had an active arthritis during the fatal attack of endocarditis.

BLOOD CULTURE

The number of bacteria in the blood stream is greater in the fatal cases. Repeated cultures of the blood from those with the milder type was usually necessary before the specific bacteria were recovered and even then the colonies were few. On the contrary, as a rule, bacteria were recovered more easily and in much greater numbers from patients with the septic type. There were, however, some exceptions to the latter statement.

The disease in several patients at first was considered of the milder type. For a few months the patients were without fever and then the disease recurred with septic manifestations and ultimate death.

The causal organism in fifty-four of the total fifty-seven patients with chronic infectious endocarditis was *Streptococcus viridans,* in one a hemolytic streptococcus of the ordinary type, in two an alpha hemolytic streptococcus.

Thirty-five of the fifty-seven patients were treated with sodium cacodylate. Three grains of a freshly prepared solution were given intravenously daily during a period of from eight to sixteen weeks. Eighteen of those so treated are dead and seventeen are living, a death rate of 51 per cent. Another group of twenty-two patients not treated serves as a comparison. Of these, seven are living and fifteen are dead, a death rate of 68 per cent. As a patient with chronic infectious endocarditis cannot be considered permanently well until two or three years after the onset, conclusions regarding the merits of any medication with this disease cannot be made until a longer period of observation has been reached.